

nature

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On location with the JET set

FUSION power is unlikely to contribute significantly to Britain's energy resources over the next 50 years. But its energy potential is so large that development of the technology must be continued. That view from ACORD, the Advisory Council on Research and Development for Fuel and Power, is contained in a discussion document, *Energy R&D in the United Kingdom* (other details, see page 535), which accords the subject barely a couple of paragraphs. But it does remark that there is general scientific agreement that the next step in the nuclear fusion programme should be the Joint European Torus (JET). And that is the rub. Everyone in Europe, not just Britain, probably agrees what the next step should be. But they differ sharply on where it should be.

What Mark Twain said about the weather, in fact, is not inappropriate to Europe's decision on a site for JET: everyone talks about it, but no one does anything about it. The long dispute has become altogether too protracted to make any sense. In February, the council of Research Ministers again postponed the decision they had already put off last December, when the new five-year European fusion programme involving JET was to be finalised. They managed agreement on initial financing of the project, but when it came to JET's location, the European Commission and Italy plumped for the European Joint Research Centre at Ispra while Britain lobbied for Culham (the design team's base), France for Cadarache and West Germany for Garching.

In an effort to reach a decision, a "fusion consultative committee" was formed, chiefly to re-examine the alternative sites but also to fulfil a more general advisory role. It was to report to the next Research Ministers' meeting, due this week (June 18). When it met in April, the committee said a decision to build JET was needed forthwith. But it did not deal with the question of the site, even though it was thought it might give its verdict early in May at a Foreign Ministers' meeting—the level at which the issue might need resolution. The agenda for the Ministers' meeting, however, was too full to include JET. By the time the consultative committee was due to meet again in May, the Italian election was in prospect and the story was already about that, again, no decision would be taken. Now the June 18 meeting itself has been postponed.

This tale of procrastination invites exasperation and

despair, not least on the part of the design team. It is understood that a formula, including financial arrangements, is now being sought which might go some way towards maintaining the team's cohesion. It is to be hoped that this materialises. Members of the team had two-year contracts which expired last December and which were renewed for six months on the now-frustrated expectation that a decision would have been reached. That did not prevent the departure of some of them then. It is not certain that it will do so now. But every effort must be made to keep these people together if Europe is legitimately to imagine itself serious about nuclear fusion.

On that ground alone an early decision seems vital. The technical assessment of each of the alternative sites is not likely to change significantly: some are better than others in some respects, others are better in other respects. There is a balance of judgment involved which it is difficult to strike because it is so fine. But the decision is essentially a political one anyway. There is no gainsaying that already complex problems are complicated further by this fact. But things have now reached a crucial stage. A healthy element of competition in scientific research is essential to progress. And that progress is only possible in the fusion field on a European scale. The danger presented by the delay over JET now threatens the prospects for nuclear fusion as a future energy source by undermining Europe's hitherto prominent position in this field in comparison with the USA and USSR.

Few people are yet saying that the USA and USSR should be allowed simply to get on with it. Those countries themselves want Europe in on the venture anyway. But there are many who, by scrambling so unedifyingly over what is admittedly a real plum and one of the most lucrative research projects of the decade, are allowing Europe's case to go by default. The Nine must now ask themselves: if the politics of petty nationalism is impairing European co-operation on fusion to the extent that no decision on a site for the project can be made, can the people of Europe really be offered much hope for the aims of the project itself? The institutions of Europe may not reveal it, but the people they serve so indirectly are the ones who are ultimately paying for the present mess. And the cost, both financial and otherwise, is growing all the time. □



The search for a Law of the Sea

THE coastal states of the world, in particular those with wide continental shelves, have for ten years now been working on certain principles: what we can grab by inventing new rules, we will claim for ourselves; what's left can be designated as a common heritage of mankind; and the profits therefrom (if there are any) can be shared amongst States Parties to the Convention (including ourselves) but (as a sop) "taking into particular consideration the interests and the needs of the developing countries". Their object has now nearly been achieved, and it looks as if we shall see in the next year or two a Convention that allocates to the coastal states a 200-mile economic zone plus almost certainly the resources of the continental margin where that margin extends beyond 200 miles. So far as scientific research is concerned, its fate within these zones will be barely distinguishable from that in the Territorial Sea, as it will be tightly controlled by a "consent" regime throughout.

What is left is known as the "Area"—the sea-bed and ocean floor and subsoil thereof (that is, not the water column) beyond the limits of national jurisdiction. These limits of national jurisdiction have not yet been defined; a number of proposals have been made, but what is common to all is that the limits are in deep water, thus ensuring that virtually all exploitable areas are kept firmly within the national jurisdiction of the coastal states.

The United Nations is becoming more adept with each decade at developing white elephants, but the possibilities opening up now as a result of the deliberations of the Third UN Conference on the Law of the Sea are even wider.

To control and supervise exploitation of the mineral sources of the Area, there will be an International Sea-bed Authority which has the potential of developing a secretariat several hundred strong with "regional centres or offices" and all the trappings of a UN specialised agency. Added to this, the Authority will have an Enterprise to carry out its activities in the Area. This Enterprise will have a "Board of Governors, a Director-General and such other officers and staff to perform such duties as the Enterprise may determine".

The task of the Authority and its Enterprise will be to extract and market mineral resources from that part of the earth's crust that is physically most difficult, and economically most costly, to exploit, with the proviso that in so

doing they must not upset the world's money markets in these commodities. As there is little likelihood of the Authority and its Enterprise becoming a profit making concern in the foreseeable future, here is a golden opportunity for the main financing member states of the United Nations and its specialised agencies to pour millions of dollars into an activity of doubtful economic or social relevance for legal-political reasons, instead of into international marine science, food programmes, fisheries and so on, which are being systematically starved of funds in the present world economic state.

The main working document of the conference is known as the "Single Negotiating Text". This first appeared at the end of the third (Geneva) session in mid-1975 and has now been revised as a result of "negotiations" during the fourth (New York) session. The size of the task before the conference (and the UN secretariat) can be realised when it is seen that the latest version of this Single Negotiating Text contains 398 Articles, as well as a number of annexes covering all aspects of the work of the conference. Many of these articles are not acceptable to countries or groups of countries, particularly the landlocked or geographically disadvantaged states which have been very active in asserting their "rights" during the recent session. They are therefore subject to further negotiation. Furthermore, a number of articles are incompatible with others developed in different committees and will need to be reconciled.

One of the mysteries of the conference has been the continued insistence of certain countries on the need to distinguish between fundamental and resource oriented research. This is a completely valueless exercise which in the long run can only do more harm to its promoters, through loss of belief in their honesty and intentions and in goodwill, than a policy of accepting Macmillan's "Winds of Change" and realising that going half way to meet the other man will pay dividends in the long run. The tactics of using a false assumption, one known to be false by the other side, as an item for negotiation are extremely doubtful. The developing countries, the policies of which are formulated by politicians notoriously only concerned with short-term results, and with no tradition of pure research, not surprisingly are unable to believe that developed-country insistence on this point is not a cover for activities which will be detrimental to them, either militarily or commercially. Quite apart

from the impossibility of formulating a true distinction, their experience of publicised episodes of this kind, even if in fact minimal in comparison with genuine oceanographic research expeditions, does not augur well for the next so-called negotiating phase of the conference.

The most important outstanding difficulties now appear to be the status of the economic zone, the regime for the deep sea-bed and subsoil and dispute settlement procedures. The USA, which is putting pressure on the conference to reach a conclusion, and whose delegation will be led at the next session by Dr Kissinger himself, has called the arrangements for dispute settlement "the glue of the whole treaty", stating that "without agreed and binding dispute settlement procedures, the treaty will not be adequate".

The President of the conference, Mr H. Shirley Amerasinghe of Sri Lanka, has proposed that the first two or three weeks of the fifth session be used for making attempts to reconcile outstanding differences on the most crucial issues involved in drafting the Convention, followed by indicative but not binding voting in the committee on the various articles in the Single Negotiating Text. Certain delegations have objected to these proposals and it is too early yet to know how the matter will be handled. There is a feeling on many sides that if the conference does not achieve a reasonable consensus by the end of the next session, it will collapse, and no Convention will be possible for many years to come. Should this happen, the consequences would be endless and could well lead to severe clashes between states on unilaterally imposed limits and restrictions which would almost inevitably be to the disadvantage of the smaller and developing coastal states, owing to their physical inability to patrol and police a 12-mile territorial sea, let alone an economic zone 200 miles or more wide.

The conference was sharply divided on the desirability of holding the next session with only a short recess, in order to keep up the momentum generated in New York, or as a further annual session in Spring 1977. In the event, after an appeal from the President, it was decided to hold the fifth session, from August 2 to September 17, again in UN headquarters, New York. It is hopefully envisaged that this will be the final working session and that it will be followed later in the year, or in early 1977, by the plenipotentiary Conference in Caracas to conclude the Treaty. □

Lithium and nuclear fusion

Can lithium reserves sustain a programme for the generation of power by controlled thermonuclear fusion?

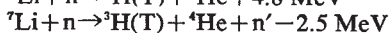
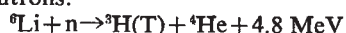
Nick Walton and Ed Spooner offer this assessment

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THE postponements by the EEC of a decision on the siting of the Joint European Torus (JET) nuclear fusion project, worrying setbacks though these are for European fusion research, should not be allowed to detract from other considerations regarding fusion power and, specifically, questions about ore reserves of lithium and future lithium demand.

Of the three possible practical methods for obtaining power from fusion, the one based on the deuterium-tritium reaction is the most favourable since the "ignition" temperature is the lowest (about 10 keV) and the average energy gain is the highest (about 1,800 per fusion reaction). The first generation of nuclear fusion reactors will therefore need a continuous supply of both deuterium and tritium fuel.

Deuterium constitutes 0.015 atom % of naturally occurring hydrogen, and is relatively inexpensive to produce from sea water. Since sea water contains about 33 g m⁻³ deuterium, the world's resources of deuterium are large (about 5 × 10¹³ tonnes) and readily available. Tritium, however, is an unstable isotope with a short half-life of 12.3 years, and occurs naturally only in trivial amounts as a product of cosmic ray bombardment in the upper atmosphere. A continuous supply of tritium for use in fusion reactors will, therefore, have to be produced artificially. The only practical method of doing this is to irradiate lithium with neutrons:



In a fusion reactor liquid lithium may be arranged as a circulating blanket surrounding the reacting plasma. Neutrons released by the fusion reactions can then be used to breed tritium fuel continuously, actually within this blanket.

The most useful of the two reactions is that involving ⁶Li, since this is exothermic and can add 4.8 MeV per nuclear reaction to the energy output of the deuterium-tritium fusion process itself. The capture cross-section of this reaction, however, peaks for slow neutrons at 0.3 MeV; it would thus use only a small proportion of the total neutrons available, since the spectrum of the neutron flux peaks at 14.1 MeV. The ⁷Li reaction is therefore impor-

tant, even though it is endothermic, because in addition to producing tritium it acts as a moderator of fast neutrons since its capture cross-section peaks at about 8 MeV. This reaction slows fast neutrons down sufficiently for them to interact exothermically with ⁶Li and produce more tritium.

A blend of ⁶Li and ⁷Li can therefore balance the opposing requirements of tritium breeding and production of additional energy. The isotopic composition of natural lithium (7.4% ⁶Li; 92.6% ⁷Li) corresponds closely to an optimum mixture; it is worth noting, however, that the tritium required by the first nuclear fusion reactors will have to be produced by irradiating concentrated ⁶Li with the slow neutrons available from fission reactors. Additionally, liquid lithium is theoretically superior to liquid sodium as a coolant. It combines such favourable properties as a low density, a high boiling point, a high heat capacity and a high heat transfer coefficient. Hence, an equally important factor which is likely to ensure that lithium will be used in nuclear fusion reactors is that it is theoretically highly suitable for use as the coolant which transfers heat from reactor cores.

Natural lithium in the form of a liquid metal blanket will, therefore, probably be essential in nuclear fusion reactors since it alone can effectively combine the three diverse requirements of tritium breeding, neutron moderation and heat transfer. Total

cumulative Western world lithium demand up to the year 2030 may conveniently be considered as the sum of four requirements, each of which can be evaluated separately (see Table 1): non-fusion use up to the year 2000; non-fusion use from 2000 to 2030; capital requirements of lithium to be used in multi-purpose blankets for fusion reactors; and lithium consumption by nuclear reactions in the liquid metal blankets of fusion reactors.

Non-fusion demand

Western world consumption of lithium in 1974 was about 3,900 tonnes metal. Demand for lithium, which is used principally as an additive to aluminium potlines, in the manufacture of glass, ceramics, greases and specialised lithium chemicals, and in welding and brazing fluxes, increased at about 5% per year in the 1960s and early 1970s. However, the unique properties of lithium are only now being exploited for highly specialised, non-substitutable applications in a variety of new and expanding industries. This has resulted in a sharp rise to a 15% annual growth rate in the last few years, a rate which is likely to continue for the immediate future at least. Over the past 65 years annual growth in demand in the United States has fluctuated considerably in response to changing economic circumstances, but has averaged about 10%.

Two major new applications of lithium can be foreseen which are likely to sustain a high rate of growth in demand. These are in high power/weight ratio batteries, and as the liquid metal coolant in fast breeder nuclear fission reactors. Annual growth in demand for lithium over the next 25 years is thus likely to be between 5%

Table 1 Estimates of Western world non-fusion and fusion demand for lithium up to the year 2030

(a) Western world lithium consumption in 1974 ¹ :	3,900 tonnes metal		
(b) Non-fusion demand up to the year 2000:			
Growth rate in demand	5%	10%	15%
Annual demand in 2000 (tonnes)	13,900	46,500	147,600
Cumulative demand by 2000 (tonnes)	200,000	426,000	958,000
(c) Non-fusion demand from 2000 to 2030, and cumulative non-fusion demand up to the year 2030:			
Demand growth rate up to 2000	5%	10%	15%
Demand growth rate from 2000 to 2030	5%	10%	5%
Annual demand in 2030 (tonnes)	60,000	811,000	638,000
Cumulative demand between 2000 and 2030 (tonnes)	923,000	7.6 million	9.8 million
Total cumulative demand up to 2030 (tonnes)	1.1 million	8.1 million	10.8 million
(d) Capital requirements of lithium needed in fusion reactors from 2000 to 2030:	~1.5 million tonnes		
(e) Lithium consumption by nuclear reactions in liquid metal blankets from 2000 to 2030:	~80,000 tonnes		
(f) Total Western world cumulative demand for lithium by the year 2030:	2.7–12.4 million tonnes		

¹ Luckenbach, W. F., *Mining Annual Review* 1975, 112 (Mining Journal, London; 1975).

and 15%. Continual development of new applications is likely to sustain a higher rather than a lower rate, and this will lead to a cumulative demand for lithium by the year 2000 of between 200,000 and 958,000 tonnes metal (Table 1). The rate of increase in demand for the years 2000 to 2030 is also likely to be between 5% and 15%. The figures suggest that cumulative Western world non-fusion demand for lithium should be between 1.1 and 10.8 million tonnes. Because of the discovery of quantitatively important new applications for lithium, it is considered that estimates in the upper part of the range are more reasonable.

Fusion demand

Future requirements of lithium for nuclear fusion reactors can be divided into two types: an initial inventory of lithium which will be used in the cooling blankets—this is estimated (Ribe, F. L., *Reviews of Modern Physics*, 47, 7-41; 1975) to need between 600 and 1,700 tonnes per 3 GW reactor; and a supply of lithium which will be used to replace the lithium destroyed by the nuclear reactions in the liquid metal cooling blankets (it is considered that this will be a relatively small quantity of about 13.5 tonnes per year per 3 GW reactor).

In order to estimate the quantity of lithium which will be needed if a

nuclear fusion programme does develop, a model calculation is used which is based on a set of postulates. These are:

- Commercial nuclear fusion reactors will start to be built in about 25 years time. For technical reasons the power generative capacity of these machines is likely to be in the 2-5 GW range, and, therefore, larger than the current largest power stations which produce only about 1 GW.

- The rate of installation of nuclear fusion reactors between 2000 and 2030 will be comparable to the planned rate of installation of fission reactors in the United States between 1970 and 2000. A lower and more likely figure for the planned amount of generating capacity to be installed by 2000 of 750 GW is taken, rather than the more normally quoted figure of 1,000 GW.

- The Western European countries, and possibly other nations, will also implement a nuclear fusion programme which will together be approximately the same size as that in the United States.

Hence, assuming a mean fission reactor size of about 1 GW and a comparable building rate for fission and fusion reactors, approximately 750 fusion reactors might be installed in the United States between 2000 and 2030. At about 1,000 tonnes of lithium per fusion reactor, this would lead to a

cumulative demand for lithium of 750,000 tonnes by the year 2030. If usage in the rest of the Western world is comparable, then cumulative Western world demand by 2030 would be about 1.5 million tonnes. This figure is comparable to the minimum estimate for cumulative non-fusion demand (1.1 million tonnes, Table 1). Cumulative demand for lithium to replenish liquid metal cooling blankets would be about 80,000 tonnes.

The total cumulative Western world demand for lithium up to the year 2030 could therefore lie between 3 and 12 million tonnes, depending principally on the growth rate of non-fusion uses.

Lithium occurrence and reserves

The concentration of lithium in the Earth's crust is of the order of 20 ppm, and in sea water about 0.2 ppm. Because of the small size of its univalent cation (ionic radius of $\text{Li}^+ = 0.68 \text{ \AA}$), lithium is an incompatible element which concentrates in the late-stage silicate melt and aqueous fluid phases formed during solidification of magmas. Crystallisation from these residual liquids has produced the unusually coarse grained acid igneous rocks called pegmatites. However, less than 10% of all known pegmatites contain suites of rare minerals, and only some of these are rich in lithium.

Table 2 Western world identified resources of lithium

Country	Location	Type of deposit	Total identified resources (tonnes Li) ¹	Identified resources ² Proven and probable reserves (tonnes Li)	Possible additional reserves (tonnes Li)
USA	Kings Mountain, N. Carolina	Large pegmatites	313,000	441,000	560,000 ³
USA	Black Hills, S. Dakota	Small pegmatites	not specified		10,000
USA	Other areas	Small pegmatites	not specified		1,000
USA	Silver Peak, Nevada	Underground brine (300 ppm Li)	553,000	454,000	2,000,000
USA	Searles Lake, California	Surface brine (70 ppm Li)	40,000	10,000	30,000
USA	Great Salt Lake, Utah	Surface brine (60 ppm Li)	707,000	not specified	not specified
USA	Salton Sea, California	Underground geothermal brine (210 ppm Li)	1,000,000		1,000,000
USA total (rounded)			2,600,000	900,000	3,000,000
Canada	Barante, Quebec	Medium pegmatites	86,000	76,000	
Canada	Bernic Lake, Manitoba	Medium pegmatites	73,000	41,000	
Canada	Other areas	Pegmatites	317,000		180,000
Canada total (rounded)			476,000	117,000	180,000
S.W. Africa	Walvis Bay	Small pegmatites	17,800	4,000	
Rhodesia	Bikita tinfield	Medium pegmatites	83,900	82,000	
Zaire	Manono and Kittido	Large pegmatites	1,000,000 (inferred)		1,000,000 ³
Africa total (rounded)			1,100,000	86,000	1,000,000
W. Australia	Coolgardie	Small pegmatites	15,000	not specified	not specified
S. America	Salar de Atacama	Underground brine	1,300,000		1,000,000 ³
Western world total (rounded)			5,000,000	1,100,000	5,800,000
				7,000,000	

¹ Norton, J. J., *USGS Professional Paper*, 820, 365-378; 1972.

² Kunos, I. A., in *Industrial Minerals and Rocks* (AIME, 1974).

³ These figures are hypothetical resources that are geographically predictable as existing in known districts.

The clinopyroxene mineral spodumene ($\text{LiAlSi}_2\text{O}_6$) is the most important lithium mineral. It occurs particularly in a small number of large unzoned pegmatites in North America. Lepidolite mica ($\text{KLi}_2\text{Al}(\text{Si}_4\text{O}_{10})(\text{F},\text{OH})_2$), eucryptite (LiAlSiO_4), petalite ($\text{LiAlSi}_3\text{O}_{10}$) and the phosphate amblygonite ($\text{LiAl}(\text{PO}_4)(\text{F},\text{OH})$) are the only other lithium minerals of economic importance. These minerals tend to occur as replacements in the central zones of smaller, zoned pegmatites which occur in Southern Africa and elsewhere.

Lithium also occurs concentrated in certain surface and underground brines. For example, the underground brines at Silver Peak, Nevada contain about 300 ppm Li, the Great Salt Lake, Utah contains about 60 ppm Li and the Salton Sea geothermal underground brine in California contains about 210 ppm Li. The Silver Peak brine is the largest known single reserve of lithium in the world and contains identified possible reserves of 2 million tonnes lithium metal. Lithium supply has recently become dominated by production from brines, and is likely to remain so in the future.

Unlike sodium and potassium, lithium does not occur in economically significant concentrations in marine evaporites because the concentration of lithium in sea water is low (about 0.2 ppm) and the solubilities of its salts are high.

For comparison with the estimates of cumulative Western world demand

for lithium by the year 2030 given in Table 1, information on resources of lithium in economically workable concentrations (ore and brine deposits) is summarised in Table 2. This suggests that Western world reserves of lithium which are identified at the present time are about 5–7 million tonnes of metal. The amount of lithium in undiscovered deposits would add a considerable, but unknown, amount to this figure. In the past there has not been a large demand for lithium and, as a result, exploration for new deposits has not been particularly rigorous or extensive. The likelihood of discovering new large lithium ore deposits, therefore, is reasonably strong.

The figure of 5–7 million tonnes for identified Western world lithium reserves is comparable to the estimate of 3–12 million tonnes for Western world cumulative lithium demand up to the year 2030. Providing that exploration for new lithium-enriched brines and ore deposits is as successful as would seem probable, it can be concluded that the Western world's resources of lithium are capable of sustaining a programme for the generation of power by controlled thermonuclear fusion. However, the fact that the figures for cumulative demand and presently known sources are similar suggests that there is no cause for the complacency shown in recent assessments (see, for example, Hubbert, M. K., *Scientific American*, **225**, 61–70; 1971, and Proceedings of the Fourth

International Conference on the peaceful uses of atomic energy (Geneva) 7, 467 (UN and IAEA, Geneva; 1972)). This is particularly the case for the European countries which possess no presently identified significant lithium resources. This fact suggests a necessity for exploration in Europe if dependence on imports is to be minimised.

Four other conclusions may be drawn:

- Non-fusion demand for lithium is likely to be greater than or equal to fusion demand.

- Demand for lithium is certain to rise, and, therefore, lithium production will have to increase quite considerably. If, for example, the total growth rate in demand were maintained as high as 10% over the next fifty-five years, as over the past fifty-five years, production capacity would have to increase by a factor of about 200.

- A large amount of liquid lithium metal must be tied up in the blanket of each new fusion plant. This represents a sudden, large increased demand equal to one-quarter of current annual consumption. This capital demand for lithium has invariably been overlooked when assessing the availability of lithium supplies for nuclear fusion.

- The future demand for lithium will be relatively inelastic since the important new uses being developed are all based upon specific and unique properties of the metal. There are, therefore, no known lithium substitutes which could significantly depress projected growth figures. □

UK ENERGY

A question of balance

The widely heralded public debate on the national energy strategy opens next Tuesday, one week after publication of the Department of Energy's report singling out the coal and nuclear industries as crucial factors in the country's energy future. Allan Piper reports

ON the premise that the best way of planning a forward march is to stand well back and view the terrain ahead, Britain's Energy Secretary, Mr Anthony Wedgwood Benn, has started mapping out the nation's energy strategy with his feet in the right place. Consider the position. The country is caught in industrial recession, and inflation is at an uncomfortable level. The demand for energy has slumped. The coal industry, committed to an ambitious expansion plan after years in decline, is producing more fuel than the country can burn, and is staggering beneath the expensive burden of record stockpiles.

The nuclear programme, years behind target and way over budget, is beset by niggling technological teething troubles, and faces powerful environmentalist opposition. Additional competitive pressures stem from the rosy opportunity for national energy sufficiency offered by North Sea oil and gas. But both must be carried through to the 1990s, when North Sea reserves will be running down and high world prices make energy imports undesirable.

It all means that when Mr Benn moves onwards from next Tuesday he will be juggling as he goes. Clutching the medium-term North Sea opportunities firmly in one hand, and manipulating nuclear options with the other, he must all the while keep the coal industry safely off the ground. Neither can he afford to drop any of the five realistic alternative possibilities—solar, wave, wind, tidal and geothermal power. Fusion is regarded as too distant. Thus, by the time the energy gap looms, the whole act must be sufficiently well coordinated to allow just one firm

step across.

Events of the past fortnight have shown that the Energy Secretary will be jostled from every side. In the build up to the coming energy forum, organisations as diverse as the coal industry, the UK Atomic Energy Authority (UKAEA) and the Confederation of British Industry (CBI) on the one hand, and Friends of the Earth (FOE), consumer groups, and trades unions on the other, have deluged his department with a welter of initial positioning statements. They indicate clearly that few of the 120 participants involved will fail to adopt attitudes largely of self interest.

ACORD report

The simultaneous publication of a long-awaited Department of Energy (DEN) report on energy research and development (R&D) strategy may help, however, to keep the energy secretary on a firm footing. Its recommendations, while arguably as predictable as those of the various positioning papers, are at least based on wider considerations. Put together by a working group of the Advisory Council on Research and

Development (ACORD), the report identifies coal and nuclear technologies, together with energy conservation, as outstanding long term R&D priorities. At the same time, it calls for the priority development of technologies to optimise the medium-term benefits of North Sea resources.

The report's conclusions are not based on the conventional method of forecasting distant energy demands, by extrapolating historical trends into the future. Instead, the ACORD working group has considered seven possible "scenarios" and suggested strategies considered necessary to meet the challenges of each one. The scenarios range from a continuation of present social, economic and technical trends until 2025, through a pattern of low worldwide economic growth, to one of high worldwide growth.

The picture that emerges suggests that whichever scenario prevails Britain is unlikely to get by comfortably without well developed coal and nuclear technologies. Even with a continuation of present trends, the report finds, the UK's installed nuclear capacity must rise to almost 25 times the existing level of 5,000 MW by 2010 if substantial energy imports are to be avoided. This is so even though the coal industry should by then have reached its expansion target of 150 million tonnes a year (25 million tonnes above present levels), since the likely decline of North Sea reserves will shift coal's greatest economic contribution to its potential as a source of gas, liquid fuels, and other hydrocarbons.

If the analyses are correct—and ACORD must feel reasonably assured of a wide response to the report's invitation for alternative scenarios from independent interests—the renewed emphasis on the crucial importance of the coal and nuclear options raises Mr Benn's greatest problem. As Walter Marshall, Chairman of ACORD and Chief Scientist at the DEN, put it last week, there is a danger that complacency in the face of adequate medium term North Sea supplies could delay major investment decisions until it is too late to retrieve the situation.

Coal

The coal industry's own solution to this thorny problem became available last week with the publication of a positioning statement for the energy debate from the National Coal Board (NCB) and coal workers' unions. It shows that the industry wants the Central Electricity Generating Board (CEGB), coal's biggest single customer, to review forward orders for coal fired or dual-fired generating plant. But as the CEGB Chairman, Arthur Hawkins, pointed out when the issue was first raised in February, his utility can

hardly pull the plug out of the North Sea market, nor the rug from under the nuclear industry, which between them should eventually supply the constant CEGB demand baseload.

Moreover, the CEGB has already given some ground to help relieve the coal industry's position. Under a payment deferral scheme it has agreed to hold coal stocks in excess of normal reserves—put at 50 days supply—so that it is now stockpiling nearly 17 million of the nation's total 31 million tonnes, a figure expected to have reached 20 million tonnes by the end of September. To offset this increase, at least in part, the CEGB will take advantage of the recent slight improvement in coal's economic competitiveness against imported oil to burn 70 million tonnes this year, an improvement of 3 million tonnes over last year's level. If the short-term investment hurdle can be cleared, the coal industry will argue, the way is open towards the targets for the future. Estimated UK reserves are put at around 45,000 million tonnes, enough to last for several centuries at current extraction rates, and the industry claims that future demand will be more than matched by production capacity.

Thus, it will be reasoned, a national energy strategy based on coal imports would be misguided. During the 1980s indigenous coal could resolve the burden of imported oil costs, and even, with the introduction of fluidised bed combustion and an increased uptake by the home steel industry, Britain could become a coal exporter as early as 1985. As to the less immediate future, the industry, like ACORD, foresees its greatest long-term economic potential in gasification and liquefaction techniques. NCB R&D spending into these fields and into integrated and remote mining systems is planned at £12 millions for this year.

Nuclear

The nuclear industry stands just as firmly behind its own position. Stressing its "excellent" safety record, the UKAEA will argue that because coal's greatest potential lies where it does there should be no major national commitment to its short term use as a generating fuel, and that the nuclear option provides the only realistic long term alternative that can be available in time and in sufficient quantity. To meet future demands, the UKAEA says, an independent R&D effort should be coordinated within the industry. The UKAEA also calls for some stability in the forward ordering pattern for power stations, while the Nuclear Power Company claims that orders must run at more than 1,000 MW a year if a rundown in construction and design work is to be avoided.



Walter Marshall: Britain's energy guru?

Recent developments on the nuclear front suggest that the CEGB might view this plea for increased nuclear capacity more favourably than it might have done even a few weeks back, in spite of its continuing pessimism over future levels of electricity demand. Last month it was announced that the troublesome Advanced Gas Cooled Reactor at Hinkley Point in Somerset is now producing Britain's cheapest electricity, at about one eighth of the cost of the most efficient fossil-fuelled station at Ratcliffe-on-Soar. Almost simultaneously, the Hinkley Point reactor obtained the long-overdue go-ahead to run up to heavy loads, and it should soon have reached 85% (about 475 MW) of designed operational output. The second reactor at Hinkley Point is also expected to come on stream soon, and it seems possible that the AGR at Hunterston on the Scottish coast could produce usable power before the winter.

Furthermore, Dr Marshall is ready to present a report on nuclear pressure vessels to the Chief Nuclear Inspector, concluding that they could safely operate in Britain without major design changes. While the finding provides an immediate fillip only to Westinghouse Electric, the US company responsible for the design and sales of the Pressurised Water Reactor, it will nonetheless indirectly boost national confidence in nuclear technology.

The CEGB may also be impressed by the ACORD analysis of the future role of nuclear power, as may Mr Benn. This indicates primarily that whichever future economic scenario is considered, an enlarged nuclear capacity seems virtually essential. The only non-nuclear scenario, that excluding nuclear plant beyond the 1980s, throws up a gloomy picture of economic growth restricted to "unacceptably low levels".

Fast Breeder

Should these favourable developments

lead to the general acceptance of plans for an enlarged nuclear technology, the vexed question of long-term uranium availability will certainly raise calls for the early introduction of the Fast Breeder Reactor (FBR), which uses uranium 60 times more efficiently than conventional reactors. The ACORD report does not consider the possible effects of an international moratorium on the FBR, but the oversight is something that the increasingly respectable Friends of the Earth, averse to the FBR in particular rather than nuclear reactors in general, may seek to amend. A government decision on whether to move from the prototype to the demonstration stage with the FBR is to be given in the autumn, and it is still possible that Britain could forego further independent development, opting instead to buy herself into other national programmes at a later stage. The CEGB is known to be engaged independently in ongoing negotiations with the French-German-Italian consortium involved in the Superphénix FBR.

Conservation

On conservation, there is general accord, with calls from both the nuclear and coal industries for tightly controlled exploitation of North Sea resources. This would not only stretch oil and natural gas reserves, but should also provide the coal and nuclear programmes with a chance to regain a firm grip on the generating market. British Gas, however, will tell the Energy Secretary next week that such measures are largely unnecessary, suggesting that further gas finds could close the predicted energy gap completely.

But, more broadly, ACORD has underlined that because of low energy conservation factors, which average around 40%, every unit of power conserved represents more than two units of primary energy. This is a proportion which may become critical in the energy climate of the late 1980s, and last week Dr Marshall announced that the portion of the DEN R&D budget set aside for conservation—included, incidentally, with the allocation for

alternative resources—is to be expanded by over £7 millions to £22 millions within the next four years.

For its part, the CBI will call for increased consumer influence in energy affairs, a theme also taken up by the National Consumer Council. On the other side of the political fence most of the unions can be expected to underline the already published call of the Amalgamated Union of Engineering Workers for complete nationalisation of North Sea resources. The AUEW also decries any extension of oil and gas use into electricity generation.

So it all begins on Tuesday with the shouting. Some may doubtless regard it as fitting that the Energy Secretary should launch his juggling act under a glare of national publicity. Others more charitably feel that if the right attitudes win through the event could be just the start of an effective exercise in "open government". Whatever the outcome of his eclectic approach one thing is abundantly clear: Mr Benn will need a cool head and steady eye to strike the necessary balance. □

NUCLEAR TRADE

Heavy water allegations

The controversy over international trade in nuclear technology and materials continues in the United States. Colin Norman reports from Washington on the latest development

THE long-simmering debate over nuclear export policies took an important new twist in Washington last week. Senator Abraham Ribicoff, chairman of the Senate Government Operations Committee, charged in a public statement that heavy water supplied by the United States to India in 1956 played a key role in India's nuclear explosives programme. Ribicoff, an outspoken critic of present US nuclear export controls, sharply criticised the State Department for failing to place strict safeguards on the heavy water sale, and he also condemned the department for failing to establish whether India used the material to produce its explosive device.

Though the State Department and the Energy Research and Development Administration (ERDA) have challenged the chief point in Ribicoff's analysis, his statement is likely to play an important part in public hearings on whether or not the United States should sell some 40,000 pounds of enriched uranium to India to fuel a large nuclear power plant near Bombay. The hearings have been postponed from June 2 to July 20.

According to Ribicoff, the United States sold India 21 tons of heavy water as a moderator for the Canadian-supplied CIRUS reactor, the source of plutonium for India's nuclear explosive. The material was supplied under an agreement that it be used only for peaceful purposes, and Indian officials have claimed that the explosive device detonated in May 1974 was produced without the need to resort to imported material.

The Indian assertion rests on the argument that heavy water in the CIRUS reactor degrades by about 10% a year, so that all the US-supplied material would have been used up and replaced by India's own heavy water (produced in a German-supplied plant) well before its explosives programme was begun. That argument was accepted by State Department and ERDA officials, who reiterated last week that they have no reason to doubt its validity. But Ribicoff claims that loss of heavy water in the reactor is much less than 10% a year, and that a substantial amount of US-supplied material was in the reactor while it was being used to manufacture plutonium.

Whatever the validity of Ribicoff's claims, the suspicion that US-supplied heavy water helped India to produce nuclear explosives is likely to be influential in next month's hearings on the sale of reactor fuel to India. Though the reactor in question, the Tarapur Atomic Reactor, is operated under safeguards administered by the International Atomic Energy Agency (IAEA), critics of the proposed sale have argued that India should accept additional safeguards on its nuclear facilities before final approval is given to the deal.

The IAEA safeguards are designed to prevent plutonium produced in the Tarapur reactor from being used to manufacture explosives. If the United States refuses to supply fuel for the reactor, however, it is conceivable that India could claim that since its nuclear agreement with the United States had been broken, safeguards on the plutonium no longer apply. According to Ribicoff, the Indian Government may be using that possibility as a "subtle form of blackmail" to ensure that the fuel sale is approved, and he argued that the United States "should seek to end the present uncomfortable and uncertain situation by exercising an option we have to buy back the plutonium generated by these power reactors".

At least one of Ribicoff's claims is, however, incorrect. He argued that the United States "never publicly acknowledged exporting the heavy water to India", but ironically, the Atomic Energy Commission trumpeted the 1956 agreement in a press release since it was one of the first nuclear deals under President Eisenhower's Atoms for Peace Programme. □

AUSTRALIA

Major appointments at stake

Peter Pockley reports from Sydney on the important jobs now open on the Australian science scene

WHILE the organisational structure of Australian government-financed science and advisory machinery is still being sorted out, the government has the opportunity of making some even more significant changes through the clean sweep of senior science appointments which have to be made between now and the middle of next year. The jobs at stake are the Chairmanship of CSIRO, the Chairmanship of the Australian Atomic Energy Commission (AAEC), and the Permanent Head (Secretary) of the Department of Science. Between them, these positions control over \$200 million of funds, well over the total of all other government research and development funds put together. Appointments to the three positions are in the gift of the government, formally by different ministers, but in practice the Prime Minister, Mr Malcolm Fraser, is expected to take a strong hand in all selections.

Dr Jerry Price, the Chairman of CSIRO, is now in his last year of office; he will reach the retiring age of 65 next March. A committee to examine the filling of the vacancy has been appointed, reportedly by Mr Fraser himself even though CSIRO reports to the Minister of Science, Senator James Webster. The committee is operating very privately, but is understood to be chaired by a leading academic. Not only has it been charged with recommending a prescription for the ideal candidate, but it is also looking critically at the structure of the CSIRO Executive and its decision-making processes.



Sir Hugh Ennor: retiring early?

CSIRO operates as a statutory authority under its own Act of Parliament, the Science and Industry Act. The Executive is the body responsible under the legislation for CSIRO's activities, and much interest attaches to appointments to the Executive and its terms of reference. The Labor Government had determined last year to allow the staff of CSIRO to elect one of the part-time members of the Executive, but the Liberals snuffed out the staff's hopes in this regard by getting elected to Government just before the election process had gone into the period of no-return. The part-timer whose retirement from the Executive was creating the vacancy for a staff member has had his term extended for a short period by the Liberal Government, thereby avoiding the need for a decision on the politically sensitive question of formal staff involvement on the boards of statutory authorities.

The CSIRO staff are making their views known to the committee on this and other matters of contention within the organisation. One proposal emerging is the possibility of restructuring the 9-member Executive (Chairman, 4 full-timers, 4 part-timers) into a "troika" of Chairman and two full-timers concerned with broad policy issues and an "outer" executive charged with administrative functions. The key to any major changes is the appointment of the Chairman. Dr Price was promoted to the Chairmanship from the Executive in 1970; this time the field is probably wide open, including to outsiders.

Sir Hugh Ennor, Secretary of the Department of Science, will reach retiring age of 65 in October 1977. Like many senior Australian Government public servants over 60, though, there will shortly be a prospect for earlier retirement with the introduction of a new superannuation scheme in July. With the Department he established in 1972 currently under threat of abolition, Sir Hugh Ennor can be expected to fight to go out with full Secretary status. Absorption of his Department within another one before his retirement would be a bitter pill, and Sir Hugh is not a man to be lightly put down.

Nonetheless, a decision will have to be made within a year about who is to head the administration of the operational branches and sections presently within the Department of Science, whether the Department exists as a separate entity or, for example, is recombined with the Department of Education. This appointment is highly

likely to go to a senior public servant. Mr Fraser has set himself against outside appointments to permanent headships following his inheritance of some of Mr Whitlam's appointees, two of whom are still languishing on the so-called "unattached list" where they draw their nice salaries and do nothing while awaiting appointments which never come.

The Chairmanship of the AAEC is vacant because of the sudden death on the job earlier in the year of Mr Bill Boswell, who had been full-time Chairman since 1972. The AAEC has been left in a very uncertain position, since both the Labor and the Liberal Governments had failed to appoint Commissioners to fill two part-time vacancies in the Commission of five. With Mr Boswell's death, the AAEC is left with only two part-timers. The previous Vice-Chairman, Sir Lennox Hewitt, is filling in as Acting Chairman while also holding down his full-time post as Chairman of Qantas. Professor Harry Messel, the other part-timer, a strong contender for the Chairmanship given more time under Labor, is probably now a non-starter on grounds of having been too closely associated with the former Labor Minister, Mr Rex Connor. If so, the appointment will have to come from outside.

The Minister for National Resources responsible for the AAEC is the Leader of the Country Party and Deputy Prime Minister, Mr Doug Anthony. A strong man who will want to appoint his own AAEC Chairman, Mr Anthony will nevertheless have to defer to Mr Fraser because of the Prime Minister's newly won influence over the whole science scene through the Australian Science and Technology Council which reports direct to him. While ASTEC does not have appointments advice within its charter, its membership does contain one or two likely contenders for the CSIRO or AAEC jobs.

Overseas aspirants, please note: the public servant pecking order in Canberra is determined largely by relative salaries, but there are some other interesting comparisons. The Chairman of AAEC currently receives 31,000 Australian dollars. Next is the Chairman of CSIRO on only \$32,500 ("only" because the CSIRO budget is roughly 10 times that of AAEC). The Secretary of the Department of Science receives \$35,000, for running an operation about a third of CSIRO's in terms of money and staff. □

● *Stop press:* Professor Donald W. George, Vice-Chancellor of the University of Newcastle, has been appointed part-time Chairman of the AAEC. The other two AAEC vacancies have also been filled.

IN BRIEF

California split

Voters in California rejected, by a margin of two to one, a proposition which would seriously have impeded—and possibly have halted—the use of nuclear power in California. The outcome was greatly influenced by the last-minute approval of three nuclear safety bills spawned by the debate over the proposition. Governor Edmund Brown signed them into law a few

days before the vote, a fact which enabled him to demonstrate his concern for safety without the risk of alienating voters by coming out in favour of the proposition.

No visit from USSR

Academician Yakov Zeldovich, winner of three Lenin Prizes and one Stalin (now State) prize for physics, was unable to attend a ceremony at Cambridge last week at which he was to

receive an honorary DSc degree. There is speculation that the Soviet authorities refused him a visa for security reasons. Yet earlier in his career Academician Zeldovich was closely connected with defence work, including research into the physics of conventional and nuclear explosions, but was not prevented from visiting London in 1965 to attend the Fourth International Conference on Relativity and Gravitation.

Do research workers know why they choose a particular subject to study? Today there are those who say that they have no right to make such a choice. It is suggested that they should rely on some "customer", backed by a committee or other collection of wise men, coming forward and telling them what they should do, and even how they should do it. While the working of this system can hardly be said to have contributed to scientific productivity, it is perhaps timely to look at the way in which those who have had the choice have exercised it. Has it generally given better results?

Several times in my career I have been fortunate enough to be able to choose my own research problems. During the 1939–45 War I was adequately supported by a rather unrestrictive research fellowship, while I was temporarily barred from military service by the register of reserved occupations. I felt that my somewhat academic studies in insect physiology had little relevance to the war, so I searched for a more practical problem. Having contacts with medical entomology, I soon found that the parasitic disease scabies, or "the itch", was causing increasing concern. Soldiers as well as civilians were suffering and were immobilised by treatment. It was also feared that any breakdown in national hygiene following enemy bombing might precipitate an uncontrollable epidemic. The disease was caused by the tiny mite, *Sarcoptes*, burrowing into the skin, and it was clear that a better knowledge of its biology was needed. I have, until recently, assumed that I decided to work on scabies for humanitarian and patriotic reasons; now I am beginning to re-examine my motives.

A later opportunity for choice occurred some ten years ago, when I was director of a research station. Provided that the station ran fairly smoothly, the staff got on with their work, and I showed up at an adequate number of committee meetings, no one objected if I did a little research in the remainder of my

time. There would have been difficulties if I had chosen an expensive field and asked for a lot of money for equipment, but I needed to show that my view that good work could be done cheaply had some foundation. So I decided to work on the mole, *Talpa europaea*. At the time I believed that I made this choice because I thought that the mole

Of mites and moles



KENNETH MELLANBY

could be a simple indicator of the potential fertility of the land it infested. Text books told us that moles were very voracious, eating twice their own weight of earthworms daily. As worms abound in largest numbers in fertile soil, I hoped that a count of molehills could suggest whether the ground was or was not worth cultivating. Unfortunately many of the well-known "facts" about moles proved to be wrong, their appetite had been overestimated, and the largest number of hills sometimes appeared in the poorest soil. The mole cannot be used to indicate fertility, but it is still a fascinating creature.

Recently, I have found myself once more involved with these two problems, scabies and the mole. At first sight they appear to have little in common, one concerning a medical pest, the other a minor pest of agriculture. I have just returned from a conference on scabies in Minneapolis sponsored by the American

Medical Association, the American Academy of Dermatology and other bodies. In order to give three lectures on the biology of the *Sarcoptes* I had to re-read my publications of over 30 years ago and check the results with recent observations. It was gratifying to find that the subject had remained virtually unchanged for this period. At about the same time as I was setting off for America a children's book which I had just written on the life of a mole appeared. I found myself comparing these two apparently very different creatures.

The itch mite is a tiny eight-legged arthropod, the mole is a warm-blooded mammal. Their main resemblance is that they both burrow, the itch mite in the human skin, the mole in the soil which could be described as the world's epidermis. The mite's burrowing causes a disease of the skin; gardeners whose lawns have suffered may think the mole gives rise to similar symptoms. But the burrowing mode of life can cause remarkable similarities in very different creatures. Both mites and moles are solitary, remaining in their tunnels for all or most of their lives. Both feed in the burrow. The young are born in the burrows, and then migrate out and eventually start their own tunnels. The males are more mobile than the females, they actively search for mates, but they separate again after copulation.

Having made this comparison, I began to examine my original motivation. Were the admirable and socially acceptable reasons I originally put forward for these studies the correct ones? Or have I some deep seated psychological urge to study the phenomenon of burrowing? In my case both investigations were reasonably productive. Should all scientists undergo psychological examinations, to fit their problems to their subconscious predilections? Perhaps research workers, to be successful, need to deceive themselves regarding their real reasons for choosing a subject, as well as those who they approach for financial support for their investigations.

correspondence

Selling the Sun

SIR,—Although man's existence depends, and has always depended, on nuclear fusion, the only functional system so far devised is some millions of miles removed and its major benefits are brought to us via photosynthesis. The "land issue" which should be faced is the fact that most surfaces are already covered by solar cells. These are called "green plants" and, at the molecular level, they can convert light energy into chemical energy with an efficiency of about 30%. Because most crops will not grow throughout the year or make best use of the available light, the realisable maximum is about 5% and in much agricultural practice may be of the order of only 0.1%. Nevertheless we are entirely dependent on this miserable conversion rate (and on past photosynthesis in the shape of fossil fuels). Is it then so far removed from reality and responsibility to suggest that we spend a little more than we do on increasing the effectiveness of biological solar energy conversion?

If terrestrial nuclear fusion becomes a reality it may make a massive contribution to our energy requirements. Photosynthesis (itself only one small item in the very large report on solar energy) does this already. Moreover, when all our oil and coal is spent it will have to serve as the major source of the elaborated carbon which we presently use unchanged or in the production of iron, plastics, fabrics and so on.

Money spent on nuclear fusion may be money well spent, but practical success is not guaranteed. Conversely, biological solar energy conversion has been with us for a long time. At the practical level it is hopelessly inefficient but there is no life without it. The room for improvement is immense. May it not even command a fraction of the expenditure on nuclear fusion?

Yours faithfully,

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Spraying controversy

SIR,—In the article by David Spurgeon entitled "Spraying controversy revived" (May 20, page 184), it is suggested that emulsifiers used in insecticide sprays used for budworm control may be linked with Reye's syndrome.

If this is confirmed, the reference to the condition being first described in 1963 following the US Air Force's aerial defoliation in Thailand and Vietnam will need to be examined critically since none of the three herbicidal agents used for defoliation in Vietnam contained emulsifiers.

Details are available in the report of the National Academy of Sciences on the "Effects of herbicides in South Vietnam", published in 1974. It may be relevant to add that this very full report contains no reference to Reye's syndrome.

Yours faithfully,

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Alternative refrigerants

SIR,—W. J. Megaw's letter (May 6, page 10) contributes to oversimplification concerning the fluorocarbon industry, and thus may mislead the incautious reader.

Refrigerants leak from refrigeration units. The causes are recognised engineering factors such as shaft seals, maintenance operations, permeation and eventual scrappage. Refrigerant half-life ranges from about 4 years for mobile and large fixed installations (non-hermetically sealed) to about 12 years for the hermetically-sealed units. The latter type is largely confined to small domestic units for sound engineering reasons.

Once a refrigerant cycle and capacity have been specified, the selection of refrigerant is made. R-22 is used where its physical, chemical and thermodynamic properties are suitable, but it is not well-suited for all uses. For this reason, R-22 accounts for only 30% of refrigerant sales rather than 50%, as suggested by Megaw. Forcing its use as a refrigerant across the board would result in major design and performance problems.

R-22 and R-12 can be manufactured in the same plant only if the plant was originally designed as a multi-purpose unit. Most fluorocarbon plants are dedicated to the manufacture of products derived from a common starting material. This approach provides the most efficient use of materials, energy and manpower, and results in the lowest-cost product.

Industry and government-supported research is under way to get the facts

in this environmental question. Recent technical developments stemming from stratospheric HCl measurements have forced further reductions in calculated ozone depletion. With these results, there is no reason at this time to implement Megaw's recommendation to sacrifice personal care products.

We all realise that our environmental decisions should be based on facts rather than emotion. Wrong "facts", however confidently presented, are not adequate.

Yours faithfully,

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Water to the Dead Sea

SIR,—It has been suggested in your columns that both electric power (November 6, 1975, page 9) and water (February 12, 1976, page 444) could easily be generated from the flow of seawater into the valley of the Dead Sea (surface at -390 m). Besides using this head for turbine generated energy, both additional turbine and electrical energy could possibly be obtained from the osmotic volume flow across a permselective hyperfiltration membrane and from the so-called dialytic battery (*Science*, **191**, 557; 1976) respectively.

Limitations to the above methods should however, be kept in mind. These include: the loss of energy due to friction and volumetric flow due to evaporation; the practical need to operate seawater hyperfiltration at about 2½ times (or more) the osmotic pressure of the feed (that is, 72.5 bar or 725 m of water); the problems of concentration polarisation (salt build-up) at the membrane solution interface reducing the effective driving force; and the fact that brackish water is available near the Dead Sea and could possibly be demineralized at a lower price than seawater.

Thus, the proposed scheme to generate electric power and potable water for the arid Dead Sea region should be evaluated in light of the above limitations.

Yours faithfully,

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news and views

New finds from Lake Turkana

from a Correspondent

ONE of the most exciting aspects of anthropological discoveries in East Africa over the past few years has been the clear demonstration of the existence of the genus *Homo* at a date much earlier than previously thought. Before these discoveries some palaeo-anthropologists had advocated a model of human evolution centring on a single, slowly evolving lineage. Although the evidence in Bed I, Olduvai Gorge and at Swartkrans, South Africa, had earlier indicated two separate, contemporaneous forms of hominid, the "single species hypothesis" continued to find favour in some circles. The discovery of KNM ER 1470 in 1972 was a crucial event in effectively challenging the one lineage concept. Subsequently, evidence of the early presence of *Homo* has been reinforced by further discoveries at Lake Turkana (formerly Lake Rudolf) and at Hadar, Ethiopia. Preliminary reports on Mary Leakey's new material from Laetoli, near Olduvai Gorge, may place the earliest evidence for the genus there at about 3.75 Myr. The lowest levels at Hadar, which may contain *Homo*, may also be near that date.

It now seems clear that the genus *Homo* existed at a very early date. It also seems clear that the taxon *H. erectus*, generally agreed to be ancestral to *Homo sapiens*, may have more antiquity than previously realised. In this issue of *Nature* (page 574), Richard Leakey reports the discovery of a new skull from Lake Turkana (KNM ER 3733); this skull bears a number of similarities with material from Peking which has been attributed to *Homo erectus*. A pelvis (KNM ER 3228), discovered stratigraphically below the skull, shows some features like those found in OH 28, from Bed IV, Olduvai Gorge. OH 28 has also been attributed to *Homo erectus*.

While the newly described material from Lake Turkana is not the earliest evidence of *Homo erectus* it may be the earliest yet found in Africa. Previously, the earliest clear African member of *Homo erectus* was the OH 9 skull from near the top of Bed II, Olduvai Gorge; this level has a date of about 1.1 Myr. The taxon has, how-

ever, been reported from the Djetis beds, in Java, in levels probably slightly younger than 1.9 Myr (Jacob, *Antiquity*, 47; 1972). Although the data of the new Lake Turkana material is not clear it would seem to have a minimum date of 1.3 Myr (Leakey and Walker, page 572 of this issue).

This strong evidence of the early and widespread presence of *Homo* poses some interesting questions. Foremost among these perhaps is the nature of the evolutionary relationships between this genus and known members of the genus *Australopithecus*. Although the classification of this Plio-Pleistocene genus is the subject of some controversy, it is generally agreed that within this group was a heavily built, "robust", lineage probably demonstrating some dietary specialisations. This group, variously called *Paranthropus robustus*, *Australopithecus robustus* or *Australopithecus boisei*, was in clear sympatry with *Homo* at several African sites. There is little agreement, however, on whether other Plio-Pleistocene hominids of a less robust morphology should also be placed within this genus. Some workers do recognise a "gracile" species, *Australopithecus africanus*, best known from Sterkfontein, in South Africa. Others, such as Robinson, however, have postulated that all members of the "gracile" type should be placed within the genus *Homo*. It now seems possible that such a two-lineage model, like the one-lineage model, is untenable. A prime species of evidence against the inclusion of all gracile hominids within *Homo* is AL 288, the associated cranial and post-cranial remains recently reported from Hadar, Ethiopia (Johanson and Taieb, *Nature*, 260, 293; 1976). This particular individual has several characters not usually included within generic definitions of *Homo*. For example, the third mandibular premolar has two cusps of very unequal size. In other hominids the premolars usually have two cusps of nearly equal size, although the third premolar in the Fort Ternan mandible (KNM FT 45), usually referred to as *Ramapithecus*, has a condition somewhat similar to that seen in AL 288. Moreover, the femur

of AL 288 has the flattened neck and lack of flare in the trochanteric region usually considered characteristic of australopithecine femora. Johanson and Taieb have in fact suggested attribution of this individual to *A. africanus*.

Such sympatry of *Homo* with one, and quite possibly two, forms of australopithecine would mean that there is little information about the direct and immediate ancestors of *Homo*. These known australopithecines, contemporary with *Homo*, obviously cannot fulfil the ancestral role. Finally, however, the coexistence of possibly three Plio-Pleistocene hominid groups may indicate, indirectly, something about the very nature of still earlier hominid evolution. When organisms initially occupy a new habitat they often experience a period of rapid adaptive radiation, so that eventually they occupy a variety of "niches" within that new habitat. During their Cainozoic history the order Primates have, in fact, experienced several vigorous radiations. It is possible that the early hominids, too, went through a period of adaptive radiation when they first began occupying the forest margin, grassland habitats in the Miocene and early Pliocene. It is possible that here, in the early Pleistocene, we are seeing the fossil evidence of such a radiation.



A hundred years ago

WE regret to hear that the strife at Sidney about the dismissal of Mr. Kreffit from the post of Curator and Secretary of the Australian Museum is not over. The subject came before the Legislative Assembly on the 6th of April, and provoked an angry discussion. Mr. E. P. Ramsay has been installed by the trustees as Mr. Kreffit's successor, and is in full work; but the Supreme Court has decided that the trustees had no real authority to remove Mr. Kreffit.

from *Nature*, 14, June 15, 1876.

Cosmic X-ray bursts

from Kenneth Brecher

COSMIC X-ray bursts, first observed by the Russian Cosmos 428 satellite in 1971 and reported in a widely overlooked paper by O. P. Babushkina *et al.* (*Sov. Astron. Lett.*, 1, 32; 1975) have now been detected by many other X-ray satellites. The ANS, SAS 3, Vela 5, and Ariel V groups have reported in rapid fire succession a range of exciting and perplexing observations of these fast transient cosmic events. In this issue of *Nature*, (page 562) J. Heise *et al.* report on their observations of "rapidly repetitive X-ray burster" MXB 1730-335 made with the Astronomical Netherlands Satellite. This object, discovered by the Massachusetts Institute of Technology SAS 3 group and reported by Professor Walter Lewin (*IAU Circ.*, 2922, March 5, 1976) is perhaps the most enigmatic "X-ray burster" found to date. Sometimes bursting at several-second intervals, at other times waiting minutes between events, its X-ray burst pattern reminds one very much of a relaxation oscillator. Indeed, as pointed out by Professor George Clark (MIT) the tight correlation found in the SAS 3 data between the X-ray burst size and the time to the following burst is reminiscent of the behaviour of a neon light bulb (rather than, for example, a flush toilet). It is as if a reservoir of energy (or material to provide it) must build up to a fixed level and discharge; the larger the discharge, the longer it takes to build up for the next firing. (In the flush toilet analogue, with filling occurring at a constant rate, the size of a burst would be proportional to the time preceding it.)

What astrophysical object could produce such behaviour? The ANS group report a position for the X-ray burster which they intersect with the SAS 3 error box: the overlap region contains what according to W. Liller (Harvard College Observatory) may be a globular cluster (a stellar system several light years across consisting of 10^4 - 10^6 stars). The importance of the possible association of the rapid burster with a globular cluster cannot be overemphasised: it may be something specific to these stellar systems as a whole, rather than the individual stars within them, which gives rise to the observed X-ray bursts. Heise *et al.* point out that at least three bursters appear to be associated with globular clusters. Nonetheless, it should be noted that at least four burst sources reported by the MIT SAS 3 group—MXB 1728-34, a source

in the Galactic anti-centre region, one in Puppis, and another in Aquilla (*IAU Circ.*, 2922, March 5, 1976, and private communications from W. Lewin)—are not obviously associated with globular clusters, or, if they are, there is no clear reason why the optical counterparts are so obscured as to be utterly unobservable. It is truly remarkable that of the 100 or so Galactic globular clusters known in the halo population the unobscured ones are not X-ray bursters, but that it is claimed that several of the obscured ones are. Perhaps a second hitherto unsuspected disk population of obscured globular clusters, or ones containing invisible stars, houses these bursters.

Making the bold assumption that it is in the nature of globular clusters to burst out occasionally, what is it specifically that gives rise to the X-ray bursts? It has been suggested for some time that a globular cluster could become unstable to the formation of a very dense or tightly bound stellar configuration at its centre. Taking this view one step further, J. Bahcall and J. Ostriker (Princeton University) and, independently, J. Arons and J. Silk (University of California, Berkeley) proposed that the steady X-ray emission observed from five globular clusters arises from accretion of intracluster gas on to a central, massive (10^2 - $10^3 M_\odot$) black hole. Adapting rapidly to the new observations, Bahcall and Ostriker have now suggested that these same black holes can also emit X-ray bursts, provided that a neutron star were orbiting the hole. Postulating an accretion disk of gas around the black hole, the neutron star would then periodically crash through the disk, slurp up some gas, and belch out the observed X-rays. This model, offered as an explanation for the recurrent X-ray burst source apparently associated with the steady X-ray source 3U1820-30 and possibly located at the centre of the globular cluster NGC6624, is difficult to reconcile with the very brief time interval between bursts from the rapid burster. Indeed, the change in mean burst period from 0.18 d (Clark, *IAU Circ.*, 2907) to 0.12 d (Clark, *IAU Circ.*, 2932) makes this model suspect even for NGC6624. The suggestion by J. Grindlay and H. Gursky (Center for Astrophysics) that the pulse shape of the X-ray bursts could arise from the scattering of a sharp X-ray pulse on a spherical cloud of very hot gas (rather than a disk) which is held down by a

massive black hole is at variance with the above suggestion. As shown in detailed Monte Carlo calculations by C. Canizares (MIT), however, the shape of the decay phase of the X-ray bursts can be well described by Compton scattering of the photons on cooler gas surrounding an object of a few solar masses rather than a massive black hole. Unless one devises one model for each source, a procedure which seems premature in view of the remarkable similarity in pulse structure from one burst source to another, one must turn from black holes to some other, more physical and realistic, model.

Most optically identified discrete Galactic X-ray sources found to date have been associated with mass exchange binary star systems, probably containing as one member a collapsed object (neutron star, white dwarf, or black hole). Various authors, such as J. Arons and S. Lea (Berkeley), have suggested that instabilities in accretion flow on to the collapsed object could give rise to rapidly variable X-ray fluxes from these objects. W. Baan (MIT) has suggested that accretion disk instabilities occurring around single neutron stars accumulating gas while moving through globular clusters could account for many of the particular properties observed in MXB 1730-335. Flash nuclear burning on the surface of a neutron star has been proposed as an X-ray burst model by L. Maraschi (Milan) and A. Cavaliere (Frascati). Other models, such as the stellar lightning model offered by Rrose Selavy (Zurich) have not been worked out in sufficient detail to make a direct comparison with observations possible at present.

Despite the fact that in the past few years black holes have been offered up as a panacea for explaining everything from the Tunguska event, to the absence of solar neutrinos, to the energy emitted by quasars, and even as a solution to the terrestrial energy crisis, there seems to be little direct observational support for the assertion that X-ray bursters provide evidence for the existence of massive black holes. If it turns out that the X-ray bursts arise as the result of one more complication in mass exchanging binary star systems, they will almost certainly lose some of their current fascination and mystery. Still, it must be said that it is really too early to tell whether the X-ray bursts are the astronomical discovery of the decade, or fluff a flash in the pan. □

Venus could have a large magnetic moment

from Peter J. Smith

TEN to fifteen years ago the only planets thought to possess internal magnetic fields were the Earth and Jupiter. But the data provided by space probes have transformed this picture—so much so that planetary fields must now be regarded as the rule rather than the exception. The strongest known field is still that of Jupiter which has a magnetic moment of 1.4×10^{30} gauss cm³. Then comes the Earth with a moment of 8×10^{25} gauss cm³, Mercury with 5×10^{22} gauss cm³ and Mars with 2.5×10^{22} gauss cm³. The Moon (admittedly not a planet), on the other hand, has no detectable internal dipole, which means to say that if it has an internal field at all the moment must be lower than 10^{19} gauss cm³.

The absence of a lunar field is explicable on the basis of the Moon's small core and its low rate of rotation. More surprising, however, especially in view of the fields discovered on the other terrestrial planets, is the apparent lack of magnetism on Venus. According to Dolginov *et al.* (*Kosmich. Issled.*, 7, 747; 1969) the upper limit of a barely detectable Venusian moment must be 5×10^{21} – 8×10^{21} gauss cm³. Yet on the grounds of size alone Venus would be expected to have a core about as large as that of the Earth (and thus larger than that of Mercury), and its rate of rotation is only about four times lower than that of Mercury. On this basis Venus ought to have a moment of 1.9×10^{23} gauss cm³, or so a forthcoming report predicts. Why, then, has a magnetic field greater than the Dolginov limit not been observed?

The first attempt to measure the global field of Venus was by Mariner 2 in 1962. No field was detected at the closest approach of 6.6 radii—a result which placed an upper limit of 4×10^{24} gauss cm³ on the Venusian moment. Five years later the flyby of the USA's Mariner 5 apparently enabled this limit to be reduced to 8×10^{22} gauss cm³, a figure derived from the observed position of the bow shock. But in the same year (1967) Russia's Venera 4, which landed on the planet's surface, measured the magnetic field directly, down to an altitude of 200 km. It was these data, combined with those from Mariner 5, which Dolginov and his colleagues used to reduce the maximum possible moment to 5×10^{21} – 8×10^{21} gauss cm³, the lowest upper limit yet proposed.

But Russell (*Geophys. Res. Lett.*,

3, 125; 1976) now claims that an alternative interpretation of the Venera 4 data is possible. Limiting his analysis to the last 10 min of the field measurements (representing altitudes of less than 5,000 km), Russell shows that for three different models of the solar wind–Venus interaction there is a strong dependence of magnetic field on altitude, with the field increasing in strength towards the planetary surface. Moreover, for two of these models the altitude dependence is close to an inverse cube law, the exception to this being the least likely model which assumes no well-developed magnetosphere. In both cases the extrapolated surface field is about 30 nT, which would be equivalent to a magnetic moment of 6.5×10^{22} gauss cm³.

In short, the altitude dependence is consistent with the existence of a planetary field, and the magnitude of the field suggests that the internal moment, if there is one, is larger than that of either Mars or Mercury. The qualification is needed here, however, because the form of the Venera 4 data, though necessary, is not sufficient to prove the presence of an internal global field. There are alternative interpretations. The magnetic observations could, for example, be explained not only in terms of an internal field but also of a field induced in the ionosphere. The rejection of one or other of the options will probably have to await further data, preferably from a Venus orbiter. But in the meantime a Venusian moment of at least 10^{22} gauss cm³ must be regarded as at least plausible and, if Venus is not to be exceptional, even likely. □

Genu valgum, copper and fluorosis in India

from Zaka Imam

FIRST reported in 1973 (Krishnamachari and Kamala Krishnaswamy, *Lancet*, ii, 877–897), a syndrome characterised by genu valgum (knock knees) and osteoporosis of long bones is widely prevalent in certain parts of Andhra, and in February 1976 some cases were also being reported from Tamil Nadu and Karnataka states in India. There are three important aspects to the occurrence of this syndrome—it is restricted to regions endemic for fluorosis, the victims are mainly under 25 yr old and there is no mention of this syndrome in the

reports by earlier workers who studied fluorosis in those regions of Andhra. The syndrome therefore seems to be a manifestation of fluoride toxicity that has emerged only during the past two decades. But the syndrome does not occur in all fluorotic regions of the states in question, nor in fluorotic regions in many other parts of the Indian subcontinent.

Although the cause of this geographical variation in the manifestation of fluoride toxicity is not yet fully understood, accumulated evidence provides some explanation. The regions where genu valgum is prevalent are mostly fluorotic regions in the vicinity of dams constructed during the past two decades. In Andhra state the syndrome is mainly confined to villages within a radius of 80 km from the Nagarjunasagar dam where there is more than 3.00 p.p.m. fluoride in the drinking water. Villages more than 50 km from the Hopset dam (Karnataka state), mostly with more than 1.00 p.p.m. fluoride in the water, have no cases of genu valgum, but villages within a radius of 5–30 km of this dam, about 50% of which have more than 1.00 p.p.m. fluoride in the water, have a few affected children. The syndrome is also prevalent in villages within 20 km of the Aliyar dam in Tamil Nadu, where the fluoride content in the water is fairly high, the minimum level being 1.9 p.p.m. The prevalence of the syndrome seems to decrease with increasing distance from the dam.

In 1974 the National Institute of Nutrition in Hyderabad suggested that the construction of large water reservoirs near areas endemic for fluorosis may be related to the development of this syndrome. The mechanism involved is not clear. It is known that the construction of large water reservoirs considerably influences the ecology of the surrounding areas by changing the soil conditions for several kilometres from the site of construction. The increased subsoil water subsequently changes the trace element composition of food grains grown on such soil and brings about changes in the trace element composition of water. The level of subsoil water in parts of Andhra where genu valgum is widely prevalent has risen in recent years. An increase in the subsoil water also increases soil alkalinity, producing conditions in which plants are known to pick up more molybdenum from the soil. In this context it is interesting that molybdenum is found in the higher range of concentrations in sorghum and rice grown in fluorotic regions. Sorghum is the staple of the poorer sections of the community and generally contains twice as much molybdenum as does

rice. Thus the poorest people, who are most afflicted with the syndrome, may be the most likely to consume a higher content of molybdenum in their diet. Copper and calcium contents of the cereals grown in fluorotic regions have been found to be within the normal range.

Earlier studies (*Br. J. nutr.*, **31**, 351–355; 1974) have shown that a relatively higher dietary intake of molybdenum can cause increased urinary excretion of tissue reserves of copper; the depletion of copper is in turn known to result in poor development of bone matrix and matrix osteoporosis. Because a principal feature of the genu valgum syndrome is osteoporosis of the lower limbs, and because it is found mostly among people whose staple food has a high molybdenum content, the interaction between the two trace elements—copper and molybdenum—seems to be important in the genesis of the syndrome. Krishnamachari (*Indian J. med. Res.*, **64**, 2; 1976) reported the analysis of water samples from 44 fluorotic villages—one sample from each village—of Andhra Pradesh, Tamil Nadu and Karnataka states. He found that “about 70% of villages affected with genu valgum had very low levels of copper in drinking water, the value being mostly below 0.01 p.p.m. None of the villages whose water contained more than 0.1 p.p.m. of copper had genu valgum although their water contained high levels of fluoride”.

This association between low copper content in drinking water and the occurrences of genu valgum supports the idea that the relationship between copper and molybdenum in total diet and drinking water could be an important factor in the genesis of the syndrome. □

Eukaryotic genome structure and function in Tehran

by Peter Newmark

An IUB-IUPAB International Symposium on Organization and Expression of Eukaryotic Genome was held at the University of Tehran on May 3–6, 1976.

THREE main themes characterised the presentations at the meeting. They were the structure of chromatin, the transcription of genes and the organisation of sequences in the eukaryotic genome.

The basic subunit structure of

chromatin was the subject of much attention. There is no doubt by now that the string of beads model for chromatin is essentially correct. Each bead or nucleosome comprises eight histone molecules, apparently two each of H2A, H2B, H3 and H4, around which is wound up to 200 base pairs of DNA. Figures given for the exact number of base pairs per monomer nucleosome ranged from 165 in fungi to 210 in chicken erythrocytes. These differences are outside experimental error and appear to be characteristic of the source of chromatin. Remarkably, in all cases the nuclease-protected DNA core of the nucleosome remains constant at 140 base pairs. This suggests that all the variability in base pair content per monomer nucleosome lies in the string between the beads which may vary with the tertiary packing induced by H1 and other, as yet unknown, proteins.

Although the way in which the nucleosome DNA is folded around the histones is not clear, the folds must be such as to account for the specific fragments that are generated when chromatin is digested by nucleases. For example H. Zachau (Munich University) presented evidence that the action of DNase II on rat liver chromatin produces 100 base-pair repeat fragments whose generation requires some degree of chromatin superstructure. Since the beads themselves are spaced at roughly 200 base-pair intervals this suggests a specific site of nuclease sensitivity in the middle of each nucleosome.

A very careful kinetic study of staphylococcal nuclease digest fragments of the nucleosome by G. Felsenfeld (National Institutes of Health) revealed that an arithmetic series of fragments of 40, 50, 60 . . . 140 base pairs is first obtained. Further digestion preferentially eliminates the 80 base-pair piece, removes four base pairs from the 130 and 110 pieces and two base pairs from the other pieces. In an extension of these studies Felsenfeld used reconstitution experiments to determine which of the histone molecules are necessary for the generation of the fragments. All the evidence pointed to the primary necessity of the H3 and H4 combination, suggesting that these two histones form the core of the nucleosome. H2A and H2B, however, are also required for the complete generation of the larger fragments that are obtained from chromatin.

The other problem of nucleosome structure is that of the arrangement of the histones. A rewarding approach to that problem has come from cross-linking studies in which chromatin is treated so as to link neighbouring histone molecules. The latest cross-

linking agents used by B. J. McCarthy (University of California, Los Angeles) avoid the possible shortcomings of earlier agents that, by their molecular size, required there to be an appreciable space between linked histone molecules. The new agents are tetranitromethane and ultraviolet light. The former crosslinks one region of H2B to H4, the latter links another region of H2B to H2A and sequential treatment of either cells or chromatin with the two agents leads to the formation of an H4–H2B–H2A trimer in very high yield.

Suggestive though much of the biochemical evidence might be, most of the participants were reluctant to reveal the model for nucleosome structure that is doubtless lurking in their minds if not in their laboratories. Not so B. Alberts (Princeton) who was happy to reveal the ‘Princeton model’ in which each nucleosome is formed by the association of a histone tetramer (H2A, H3, H4, H2B) on one of the DNA strands with an identical tetramer on the other strand. The entire nucleosome particle has a dyad axis of symmetry and undergoes an allosteric transition to an open form to allow genetic readout without histone displacement. In spite of its attractive features the ‘Princeton model’ is still largely speculative and has yet to face a critical experimental test. Its ultimate fate will probably only be determined when it becomes possible to study nucleosome structure by X-ray diffraction. That may not be too far off to judge by a recent Russian success in producing crystals of the 140 base-pair nucleosome core.

Beyond that is the question of the superstructure of the nucleosome string. E. M. Bradbury (Portsmouth Polytechnic) described neutron diffraction patterns of chromatin in which the 10.0-nm peak, believed to represent the internucleosome spacing, was not truly meridional. That rules out a linear arrangement for the string in favour of a flat coil that would contain six nucleosomes per turn. A solenoid structure, probably left-handed, was also favoured by F. Crick (MRC Molecular Biology Laboratory, Cambridge) who speculated further that a linear strand of naked DNA might sometimes be found running through the axis of the solenoid.

The problem of understanding transcriptional mechanisms in eukaryotic systems can be approached in a variety of ways. A basic question is whether one can mimic *in vitro* the natural process. Many experiments indicate that with bacterial or eukaryotic polymerases it is possible to achieve some fidelity of transcription as revealed by the detection of specific mRNA sequences. Whereas J. Paul (Beatson

Institute, Glasgow) emphasised the technical difficulties involved in these experiments, R. C. Huang (Johns Hopkins University) described a system in which many of them seem to have been overcome. In particular she described data on the transcription of kappa chain RNA from mouse myeloma cells. Using methods that limited chromatin damage and specifically selecting out only newly synthesised molecules to avoid the complicating factor of endogenous messengers, she demonstrated the faithful transcription of the single-copy Kappa chain gene. Furthermore, using cDNA which had been reverse transcribed from the kappa chain messenger, it was possible to show that considerably more of the kappa message was synthesised from the chromatin isolated from a kappa chain-producing cell line than from that isolated from a lambda chain-producing cell line. In other words selectivity of transcription can be mimicked *in vitro*. McCarthy presented evidence that generalised that concept. In a study of transcription from *Drosophila* chromatin he was able to show that the most abundant sequences present in *Drosophila* heterogeneous nuclear RNA are also those that are preferentially represented in the transcript obtained from *Drosophila* chromatin *in vitro*.

What factors control selective transcription from chromatin? According to W. J. Rutter (University of California, San Francisco) at least some of the selectivity resides inherently in RNA polymerase. The strongest evidence for that claim comes from his evidence that the transcription of naked yeast DNA by RNA polymerase I in the presence of magnesium ions resulted in a transcript that was specifically enriched in ribosomal RNA sequences. The same may apply to transcription by RNA polymerase III but, in contrast, both yeast RNA polymerase II and *Escherichia coli* polymerase produce largely non-specific transcription on yeast DNA.

Transcriptional selectivity is also determined by chromatin-associated, non-histone proteins. One particular fraction of these proteins, described by A. J. MacGillivray (Beatson Institute) seems to contain a putative regulatory protein, but to isolate it at present would be a task equivalent to hunting for a needle in a haystack. E. W. Johns (Chester Beatty) described a different class of proteins known as HMG proteins, some of which are being physically characterised by K. Javaherian (Tehran University). The HMG proteins are histone-related and probably play a structural rather than regulatory role in the chromosome.

As though to demonstrate the lack of understanding of transcription in

eukaryotic systems, M. Ptashne (Harvard) was allowed to display the advanced state of the art in bacteriophage λ . For example, his sequencing data explain why a small repressor transcript is associated with the synthesis of small amounts of repressor whereas a larger transcript is associated with increased synthesis. It seems that the two transcripts are produced in approximately equal amounts at different times of infection but the smaller transcript, in contrast to the larger one that is initiated 2,000 base pairs to the right of the coding sequence, does not contain a ribosome binding site and is therefore translated with a relatively low efficiency. Thus this simple virus makes use of both translational and transcriptional controls to modulate synthesis of its repressor protein.

The general organisation of gene sequences in eukaryotes was outlined by E. H. Davidson (California Institute of Technology). Most recently he and W. H. Klein (California Institute of Technology) have been able to produce evidence in favour of their hypothesis that the 300 base-pair repetitive sequences that are interspersed between the structural genes are involved in the control of expression of the structural genes. Only 10–20% of the 5,000 or so families of repetitive gene sequences in the total sea urchin genome were found adjacent to the particular structural genes that are expressed in the gastrula stage. That result indicates that particular repetitive sequences are associated with, and may well therefore control, particular structural genes.

Evidence for a specific example of a controlling region directly adjacent to a structural gene was provided by A. Chovnick (University of Connecticut). He has found a *cis*-acting *Drosophila* mutant at the rosy locus in which there is increased production of xanthine dehydrogenase activity which cannot be accounted for in terms of an alteration in structure, stability or sensitivity of the enzyme. The increase in production must therefore be due to the alteration of a controlling region and Chovnick is able to map that mutation within 2–3 kilobases of the well-defined structural gene for xanthine dehydrogenase. He has recently collected new mutants of this type and their mapping is in progress.

The organisation of gene sequences in *Drosophila* is, however, not without its surprises. M. Young (Stanford) describing recent work on the cloning of DNA fragments, reported the occurrence of multiple rather than single copies of two genes coding for abundant mRNA species in *Drosophila*. In one case the gene, which codes for a heat shock-induced protein, was found to be locally repeated in multiple tandem blocks separated by spacers. In

the other case the gene, probably as a single copy, occurs at 33 different sites scattered throughout the genome. Previous data suggested that such repeats would be unusual and it may therefore turn out that these results are unique for genes making abnormally abundant RNA species.

The most complete evidence for gene organisation in eukaryotes still comes, for technical reasons, from those genes that occur in multiple, tandem copies. An excellent example was provided by M. Birnstiel (Zurich University) who has thoroughly analysed the organisation of the histone genes in the sea urchin. The genes, separated from one another by spacers, are on one DNA strand in the order H4, H2B, H3, H2A, H1 and are transcribed as a single unit starting with the H4 gene.

It is clear that the basic structure of chromatin will shortly be known. That, together with recent technical advances in DNA sequencing and cloning, bodes well for our better understanding of the structure and function of the eukaryotic genome. □

Galactic cannon for shooting out radio sources

from John Gribbin

THERE seems to be a clear division in the Universe between the double radio sources associated with many elliptical galaxies and the more spherical outward blast of material from such spirals as Seyferts, even though both kinds of violent event are believed to be produced by explosions in the cores of the respective galaxies. Now, R. H. Sanders has presented calculations of confinement of magnetic field and relativistic particles in a plasma produced in such a way by ram pressure; he shows that just these two kinds of explosion can occur, and that the difference depends on the presence or absence of a massive central source—essentially, a point mass at the galactic nucleus (*Astrophys. J.*, **205**, 335–345; 1976).

The production of paired radio sources, moving outwards on opposite sides of the nucleus of an elliptical galaxy, can also be explained by other models, such as collimated beams of relativistic particles or the fragmentation and ejection of massive objects from the nucleus. Sanders makes no claim that his calculations represent the one true approach, but the success of these numerical simulations is striking.

ing, and may encourage some further thought on just how satisfactory the alternative models are. In essence, the problem Sanders has tackled is to find a mechanism for confining the ejected plasma within a narrow cone (vertex angle 12° to 25°); in earlier work with Prendergast (*Astrophys. J.*, **188**, 489–500; 1974) he has already looked at the opposite limitation, when an explosion occurring in the nucleus of a disk of gas (representing a spiral galaxy) can affect gas motions in the plane of the galaxy far from its centre, as opposed to the puzzle for double radio sources of how the emission is confined along the rotational axis.

Within the plane of such a hypothetical disk, expanding plasma produced in a central explosion meets a large mass of material which is pushed along, as Sanders graphically puts it, like a moving snowplough. In the direction of the rotation axis, above and below the plane, the explosion can soon break out into a region of lesser density, and this is obviously of interest to the confinement problem. And, happily in view of the observations, it turns out that the conditions for explosions being able to break out of the plane and for explosions affecting the plane itself over a large distance (essentially depending on the thickness of the disk) are mutually exclusive. But even so, some extra refinement is needed to confine material from explosions which do burst out within the very narrow cones observed in elliptical galaxy radio sources.

Sanders assumed first that there is a constant stellar density core in the nucleus of a giant elliptical, with the gaseous disk structure hypothesised as lying within this core. For a constant thickness disk, an explosion breaking out from the core still expands over almost the entire hemisphere, but this can be overcome by introducing non-uniform conditions, in the form of a massive central point mass. This has two effects. First, the disk is much thinner near the central mass, producing a shape which Sanders likens to a cannon barrel; second, this "barrel" shapes the blast wave from any central explosion, with a degree of focusing just in the required range of 25° and lesser vertex angles.

The implications of this study extend well beyond the "simple" study of radio astronomy, hinting at fundamental differences which may explain the division of galaxies into spirals and ellipticals. There are no Seyferts—indeed, no known spirals—with the double radio source structure typical of radio ellipticals, and on Sanders' evidence the reason may simply be that spirals do not have large point masses at their centres. One school of thought holds that elliptical galaxies could have

formed in the expanding Universe on the "seeds" of just this kind of central massive object, in the form of a black hole or "retarded core", while spirals formed from collapsing gas clouds in a quite different process (see review in *Nature*, **252**, 445–450; 1974); opponents of this view may of course take the present results as indicating a fatal flaw in the rammed plasma model! But even if we now have a better understanding of the means by which an explosion at the centre of a galaxy can be colimated to produce a double radio source structure, the \$64,000 question remains: what is the mechanism of the explosion itself? The energies involved hint very strongly at a gravitational process of some kind, and this again suggests the presence of massive central objects. □

Solar-geophysical exchanges discussed in the Soviet Union

from Roger H. Olson

FROM May 17 to 25, a joint US-Soviet conference was held at Kiev University and the Hydrometeorology Service in Moscow to plan for joint research and exchange of data in the fields of solar-geophysical activity prediction, in which the Crimean Astrophysical Observatory is prominent, and the effects of solar-geophysical disturbances on weather and climate. About 30 scientists and specialists attended, representing such subjects as solar physics, geomagnetism and meteorology.

There is much precedent for co-operation in the exchange of solar-geophysical forecasts and data. For years such cooperation has existed because of the need to consider solar activity in radio warning services. The need for cooperation has been emphasised more and more recently because of the need for radiation warnings to space flight operations and because of various other operational problems. In the United States daily forecasts of solar flare and proton events and of geomagnetic activity are available from the National Oceanographic and Atmospheric Administration Space Environment Forecast Center in Boulder, Colorado. In the Soviet Union, such forecasts, particularly of solar activity, are made on demand at several institutions. It was agreed that in the near future daily forecasts would be made from both sides and exchanged in real time, so that each could benefit from the thinking of the other. A common system of forecast

verification, perhaps based on probability forecasting, will be devised. And efforts will be made to agree on a limited list of indices of solar and geophysical activity, to help avoid confusion.

A system of exchange has been set up between the two countries, under which scientists or technicians from each side can spend several months in the other country, working in more detail on the types of problems discussed at the conference. The first such exchange has already been concluded, with L. Svalgaard of the USA visiting the USSR, and V. Loginov visiting the USA. The next exchange will be in the spring and summer of 1977, and will involve personnel interested in solar forecasting and data exchange.

The language barrier at the conference was minimised by the presence of expert interpreters and the fact that most of the Russians were conversant with English. But they were not aware of recent results published in English language journals such as *Nature* and *Science*, and the Americans were not familiar with the Russian scientific literature, except that which has been translated into English. □

Liposomes to lysosomes

WITH regard to the article "Liposomes to lysosomes" (*News and Views*, **260**, 749; 1976) the statement that "A non-antigenic vesicle would . . . prevent the enzyme from eliciting an immunological response . . ." is not necessarily correct. Far from preventing an immunological response there are convincing demonstrations in the literature that liposomal entrapment of antigen can augment antibody production. In other words, liposomes can act as immunological adjuvants. This was first described by Allison and Gregoriadis (*Nature*, **252**, 252; 1974) using diphtheria toxoid as the antigen and again by Heath *et al.* (*Biochem. Soc. Trans.*, **4**, 129; 1976) using bovine serum albumin. While it may be possible in the future to minimise the response to a given antigen entrapped in liposomes by a suitable choice of lipid composition, charge or size range, the fact that non-antigenic vesicles can increase the immunological response to an antigen remains an incontrovertible fact.

D. CAIRD EDWARDS

articles

Permafrost oxygen isotope ratios and chronology of three cores from Antarctica

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Permafrost core sediments, associated with the last intrusion of the Ross Ice Shelf in the New Harbor region, were deposited in marine (0–85 m deep) as well as freshwater environments (100–125 m). Oxygen isotope ratio measurements on these cores provide palaeoclimatic information and show that the extension of the Ross Ice Shelf predates 150,000 yr BP, whereas the radiocarbon date of its retreat is about 5,800 yr b.p.

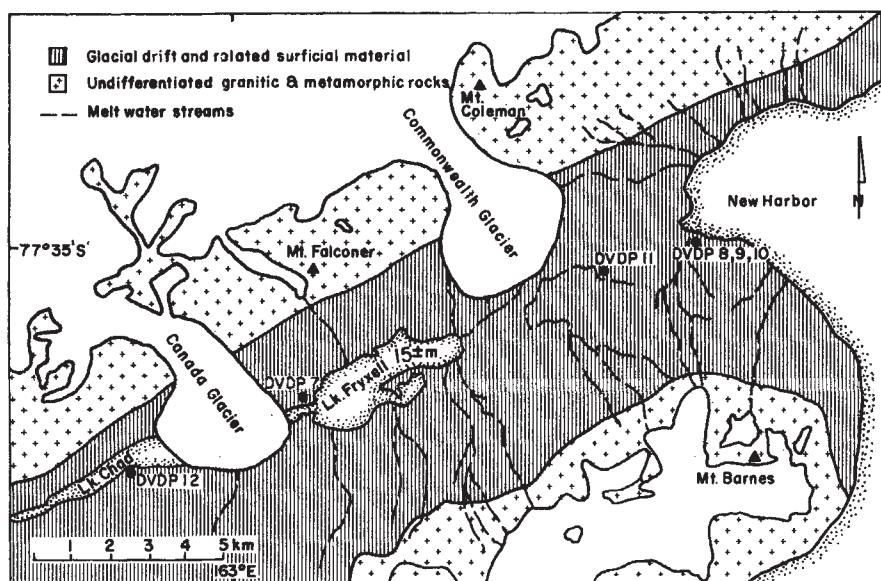
THE Dry Valley Drilling Project (DVDP) was designed to study the physical, chemical and biological regimen of the subsurface in the arid and ice-free valleys¹ of the McMurdo Sound area of Antarctica. Several sites were cored in Taylor Valley (Fig. 1); this report discusses the oxygen isotope results for cores 8, 9 and 10 drilled near the shore of New Harbor at the mouth of Taylor Valley in the austral summers of 1973–74 and 1974–75. Cores 8 and 9 are from the same site (77°34'37"S, 163°31'00"E). Core 10 is from nearby at 77°34'43"S, 163°30'42"E. The primary core is from hole 8, which extends vertically to a depth of 157.06 m below the surface. Hole 9 was drilled next to hole 8 to a depth of 38.34 m, to obtain a more complete record of an interval of poor recovery of core 8. Hole 10 was

drilled in an attempt to penetrate sediments below 157 m; it was terminated at a depth of 206 m when marine groundwater under hydraulic pressure rose up into the hole and froze. The surface elevations of holes 8 and 9 and hole 10 are, respectively, 1.9 and 2.8 m above sea level.

During the past decade there has been a substantial increase in the use of oxygen isotope ratios as a climatic indicator (for instance, the pioneering work on the Camp Century, Greenland ice core²). This use is based on the temperature dependence of oxygen isotope fractionation in condensation processes. Although such fractionation follows simple rules for well controlled experimental processes, the interpretation of the ratio changes in nature is more complicated. A change in isotope ratio may be caused, for instance, by either one of the following: (1) change in mean annual temperature, (2) shift in seasonal temperature distribution and precipitation amounts and (3) change in altitude³. With a proper evaluation of these factors, a climatic record of the last glacial cycle (~ 100,000 yr) can be obtained for the Arctic and Antarctic ice sheets.

Permafrost cores can extend this record to older glaciations. Of course, the interpretation is more complicated than for ice cores because the accumulation of sediment is not necessarily

Fig. 1 Location map of DVDP drill sites Lower Taylor Valley (after ref. 9).



continuous. In addition, a portion of the ^{18}O record may be missing and replaced by 'younger' water because the temperatures were perhaps not always sufficiently low for permafrost formation, or because post-depositional melting erased part of the record. Our work on permafrost ice gives an unequivocal ^{18}O record of the core waters. The geological and climatic information should, however, be derived mainly in conjunction with other parameters.

The New Harbor holes are close to the shoreline and the upper sediments were deposited in a deltaic environment. Any shift from a marine to a continental environment should be accompanied by a change in oxygen isotope ratios because meteoric waters are, in general, depleted in ^{18}O in comparison to seawater. For example, ice at Byrd Station contains $\sim 4\%$ less ^{18}O than undiluted seawater. Brackish waters, composed of a combination of undiluted sea- and freshwater, have intermediate values of ^{18}O deficiency. The ^{18}O ratios of brackish waters depend on the relative portions of undiluted seawater and freshwater and on the ^{18}O content of the freshwater. It is possible to derive the percentage of freshwater dilution and the palaeosalinities from the core isotope data, provided a reasonable estimate of the oxygen isotope content of the freshwater source can be made.

Experimental procedures

The permafrost core sections were kept frozen after collection from the bore hole. They were stored temporarily at McMurdo Station and at present are in the Antarctic Core Library at Florida State University, Tallahassee. Portions were packed in dry ice, shipped to Seattle and stored in a cold room of the Quaternary Research Center until analysed. For analyses, a frozen section of the core with a diameter of 6.2 cm and a thickness of ~ 4 cm was put in vessel A in Fig. 2. The basic

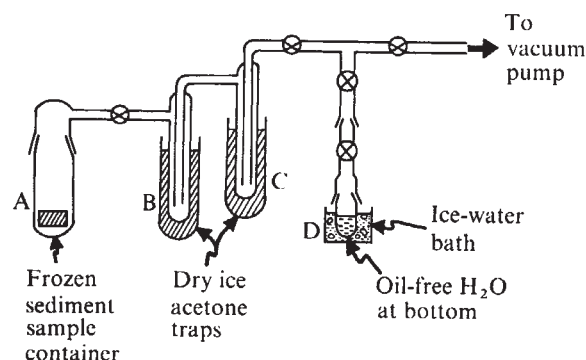


Fig. 2 Apparatus for water extraction (vacuum distillation).

purpose of the apparatus shown in Fig. 2 was the complete extraction of meteoric waters from the sediment. After evacuation of vessel A (through traps B and C cooled with a dry ice-acetone mixture), the ice in the sediment was allowed to melt at room temperature and the water was distilled into traps B and C. Evacuation took only several minutes, during which time the sample remained almost entirely frozen. To ensure 100% recovery, we allowed a minimum of 12 h of vacuum distillation. Unfortunately, not only water but also diesel fuel, used as a drilling fluid (DFA), was collected in traps B and C. The resulting emulsion, evidently formed during the early stages of vacuum distillation when transfer was rapid, made it impossible to separate the fuel from the water. Slow condensation in trap D (kept close to 0°C) yielded water and a floating layer of oil that was removed with the aid of a separatory funnel. The water obtained was equilibrated with CO_2 gas at 25°C according to standard procedures.

The $^{18}\text{O}/^{16}\text{O}$ ratios of the carbon dioxide were measured with a Nuclide 6-60 mass spectrometer.

The oxygen isotope ratios are expressed as $\delta^{18}\text{O}$ according to

$$\delta^{18}\text{O} = \frac{(^{18}\text{O}/^{16}\text{O})_s - (^{18}\text{O}/^{16}\text{O})_{\text{SMOW}}}{(^{18}\text{O}/^{16}\text{O})_{\text{SMOW}}} \times 1,000\text{‰}$$

where S denotes sample and SMOW standard mean ocean water. It is clear from the above definition that marine waters as a whole have $\delta^{18}\text{O}$ values near 0‰ .

^{18}O results and chronology

Oxygen isotope ratio measurements of snow accumulating on the McMurdo Ice Shelf yielded δ values from -32 to 34‰ (ref. 4) and from -28.9 to -31.9‰ (ref. 5). Ice samples collected by us from this ice tongue, however, gave δ values of -1.6 and $+2.0\text{‰}$, because ice within the tongue is derived largely from freezing of seawater and not from snowfall. Surface δ values of Ross Ice Shelf snow at Little America ~ 800 km from New Harbor seem to be near -20‰ (ref. 3). Meltwater from the Lower Wright Glacier, forming the surface water of Lake Vanda in Wright Valley, has a δ value of -29.5‰ (ref. 6). Our own measurements include two samples from Hobbs Glacier, ~ 40 km south-west of New Harbor with δ values of -28.1 and -25.0‰ , and one sample from Garwood Glacier, ~ 50 km south of New Harbor with a δ value of -26.6‰ . Ice preserved from the last sheet to ground in McMurdo Sound has δ values ranging from -11.4 to -35.3‰ , because ice in this sheet originated both from snowfall and freezing of seawater. Lower δ values are encountered for major glaciers fed by high altitude polar plateau ice. Two of our samples from Walcott Glacier (~ 70 km south of New Harbor) gave a δ value of -37.8‰ ; and a value of -40‰ was given for Taylor Glacier at the head of Taylor Valley (C. H. Hendy, personal communication). From this summary, it seems that current atmospheric precipitation yields $\delta^{18}\text{O}$ ratios of $\sim -28\text{‰}$ near New Harbor. Runoff from low-altitude valley glaciers also should have this approximate δ value. Meltwater from Taylor Glacier could, however, introduce lower δ values of $\sim -40\text{‰}$.

It is more difficult to estimate the $\delta^{18}\text{O}$ values of freshwater during the coldest interval of the last glaciation. The Byrd Station ice core shows a lowering of $\sim 6\text{‰}$ in δ values⁷. Thus a reasonable guess for $\delta^{18}\text{O}$ values during this episode would be -34‰ for local precipitation near New Harbor and -46‰ for nearby polar plateau ice.

The $\delta^{18}\text{O}$ values obtained for permafrost waters in the New Harbor cores are plotted in Fig. 3 against core depth. The δ values are all relative to the SMOW standard. The classification in Fig. 3 of sand, gravel, and diamicton units is based on the core descriptions^{8,9}. Further studies will undoubtedly provide additional detail, but this is not critical for our discussion.

The oxygen isotope ratios of the permafrost waters give the following picture: (1) Fully marine, or nearly fully marine, conditions down to 85 m; (2) a transition zone to a much less saline environment between 85 and 100 m; (3) freshwater, with possibly some addition of seawater, between 100 and 125 m. The lowest δ values ($\sim -23\text{‰}$) are encountered in the sand layer at a depth of 112–114 m. If the freshwater component for this layer was derived solely from local Taylor Valley waters, one would expect δ values of -28 to -34‰ . Seawater dilution of respectively 18 and 32% would increase these values to -23‰ ; (4) a transitional zone from fresh to brackish water ratios between 125 and 130 m; (5) brackish water between 130 and 155 m, with δ values ranging from -7 to -14‰ . A mixture of two-thirds seawater and one-third freshwater ($\delta^{18}\text{O} = -30\text{‰}$) would yield the average δ value of -10‰ ; (6) the return to near freshwater values between 150 and 184 m. The lowest δ values, $\sim -20\text{‰}$ are comparable with the ^{18}O values encountered in the 100–125 m interval.

The above calculation of palaeosalinities assumes freshwater contributions of local origin. It is, however, possible that meltwater was derived from Ross Sea ice transported over some distance. As mentioned, higher $\delta^{18}\text{O}$ values would result and little or no seawater dilution would be needed to explain the δ values for episodes (3) and (6).

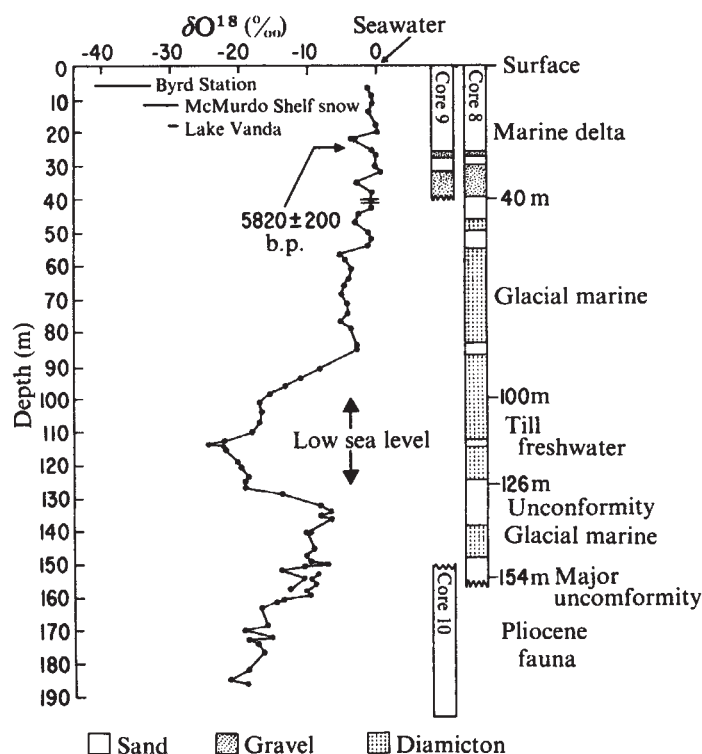


Fig. 3 $\delta^{18}\text{O}$ ratios relative to the SMOW standard of permafrost waters in cores 8, 9 and 10. The ^{14}C age at 23 m was obtained for shells in cores 8 and 9.

There is considerable evidence for a succession of grounded Ross Sea ice sheets in the McMurdo Sound region. The most recent grounded ice sheet in McMurdo Sound was not fed by East Antarctic ice dammed behind the Transantarctic Mountains in the dry valley region, nor by local glaciers, but rather by grounded ice that flowed into the sound from an area to the east and south-east that is now covered by the Ross Ice Shelf¹⁰. The surface contours of the youngest ice sheet occupying McMurdo Sound can be reconstructed from its widespread moraines and drift. Drift associated with the most recent grounded Ross Sea Ice Sheet is also encountered at New Harbor (G. H. Denton *et al.*, in preparation). Thus the upper portion of the core is associated with depositional features of the Ross Sea Ice Sheet and its associated retreat.

Limiting ages for the recession of grounded ice can be obtained from shells collected in silts near the upper marine limit in the McMurdo region¹⁰. At the mouth of Taylor Valley, New Harbor, raised silts occur up to an elevation of at least 7.5 m. *Adamussium colbecki* shell samples collected at or near the marine limit gave dates of $5,200 \pm 70$ and $4,950 \pm 70$ yr b.p. (QL-137 and 138; QL=Quaternary Isotope Laboratory at the University of Washington). Likewise, similar shell samples from raised marine deposits at nearby South River gave ^{14}C ages of $5,550 \pm 70$ (QL-72) and $5,500 \pm 60$ (QL-96) yr b.p. Moreover, shells preserved in the McMurdo Ice Shelf date back to $5,750 \pm 60$ yr b.p. (QL-166). These results imply the recession of the youngest Ross Sea Ice Sheet from New Harbor before 5,800 yr b.p.

In calculating all ages of marine shells in this paper a 850-yr

correction was applied for ^{14}C ocean water deficiency in McMurdo Sound. This correction was obtained by dating living *A. colbecki* shells collected from near New Harbor in 1974 (850 ± 45 yr b.p.; QL-98). Deeper circumpolar waters have a maximum ^{14}C deficiency of 16%; equivalent with an 'age' of 1,400 yr (ref. 11). Exchange with atmospheric CO_2 will reduce the ^{14}C deficiency in surface waters, and the 850-yr correction seems a reasonable value for this region. This correction is the smallest possible, as nuclear bomb ^{14}C may have increased surface water ^{14}C levels.

Two *A. colbecki* shells in cores 8 and 9, at the 23-m level, gave a ^{14}C age of $5,820 \pm 200$ yr (QL-191). Evidently the top 20 m of sediment has been deposited since the retreat of the Ross Sea Ice Sheet from the New Harbor region. Moreover, it seems likely that the upper 40–50 m of sands and gravels were deposited in a short interval during, and subsequent to, the recession of the Ross Ice Sheet. This opinion is buttressed by a ^{14}C date of $4,650 \pm 70$ yr on shells from a bed located 200 m south (QL-161). In our opinion, the top 40 m of sediments have been deposited within the last 10,000 yr. Deposition was in fully marine conditions, as indicated by the ^{18}O data (Fig. 3).

The contacts between diamictons and the stratified water-laid sand and gravel beds are sometimes gradational and do not seem to be erosional. For this reason, the diamictons are considered to be water-laid⁸. The permafrost waters of the diamicton between 54 and 82 m are nearly fully marine, and the diamicton must have been deposited in seawater through ice rafting.

A fully grounded Ross Sea Ice Sheet possibly could have prevented penetration of seawater to New Harbor, a situation that might produce freshwater ^{18}O ratios in the permafrost. Perhaps this is the reason for the low $\delta^{18}\text{O}$ ratios encountered in the diamicton at 87–123 m. On the other hand, it is quite possible that the sea level was lowered sufficiently for emergence of the New Harbor site. Upper marine limits in this area indicate a depression of at least 7.5 m. With a total sea level lowering of ~ 140 m, freshwater δ ratios would be expected somewhere near, or above, the 130 m depth in the core. It is possible that the 125–100 m section with its freshwater permafrost ratios was deposited during such an interval of low sea level.

Few conclusions can be drawn from the ^{18}O record below 125 m. The slight geographical difference in coring sites 10 and 8+9 complicates matters. The overlap in δ values around 154 m (Fig. 3) indicates little difference between the cores, but this may be coincidental.

It is tempting to assign an early-late Wisconsin chronology to the core, with an age of $\sim 20,000$ yr at a depth of 120 m. Palaeomagnetic work on core 10 seems, however, to indicate an age of several million years for the bottom portion of the core¹². In addition, foraminifera from DVDV holes 8, 9 and 10 indicate an appreciable older age¹³. These results may be caused partly by the reworking of sediment. Faunal assemblages between 18 and 35 m could perhaps indicate a middle to late Pliocene age range¹³, but our ^{14}C age clearly shows this interval to be of Holocene age. In this instance, older Ross Sea sediment has been transported to the New Harbor site through the action of the Ross Ice Sheet. This leaves the possibility of more core sections with reworked materials. A solution to this problem may be coming from additional palaeomagnetic determinations.

Potassium-argon dates on basalt flows that occur in the southern area of McMurdo Sound and that predate the last extension of the Ross Sea Ice Sheet show that the last ice sheet growth postdates 150,000 yr (G. H. Denton *et al.*, in preparation). The fabric and lithology studies of S. C. Porter and J. Beget in the Quaternary Research Center suggest that the thick diamicton near the 100-m depth probably represents grounded glacier ice of Ross Sea origin whereas other diamictons in the core probably are of glacial marine origin. Again one would infer a short chronology if this episode was tied to the last expansion of the Ross Sea Ice Sheet. In our opinion, sediment deposition above the 126-m depth is restricted to the last 100,000 yr. A major unconformity is suspected near 154 m.

Foraminiferal composition below this depth indicates a Pliocene age¹³.

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Quarks in the early Universe

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The quark theory of hadrons provides a valuable tool for interpreting conditions during early epochs of the Universe. In particular, an asymptotically free theory of quarks seems to rule out the possibility that the present-day entropy of the Universe could be due to dissipation of low level fluctuations. Apparently either the early Universe was inhomogeneous on small mass scales or anisotropic.

It is by now almost a certainty that hadrons consist of quarks bound by vector gluons¹. The fact that free isolated quarks have never been observed evidently means that in ordinary conditions quarks are permanently bound inside hadrons. There are, in fact, reasons² for believing that the force between quarks increases with distance rather than falling off as electromagnetic forces do. On the other hand, high energy electron-proton scattering experiments suggest¹ that when the separation between quarks is $< 10^{-13}$ cm, then the force between the quarks is rather weak. Thus one might guess that when the density of matter is increased to the point where the mean distance between quarks in different hadrons is $\ll 10^{-13}$ cm, then the hadrons will disappear and matter can be described as quark 'soup'³.

Equations of state

If one accepts the idea that matter consists of free quarks above a certain density then it becomes possible to work out the properties of matter at superhigh densities. For example, the energy density of matter at superhigh densities will have the form

$$\rho = An^{4/3} + B \quad (1)$$

where n is the baryon number density and A and B are constants. The constant A depends on the quark-gluon coupling constant g and entropy per baryon; at zero entropy⁴

$$A \simeq 9/4(3\pi^2/\kappa)^{1/3}[1 + (8g^2/3\pi)]\hbar c$$

where κ is the number of quark 'flavours' contributing to the energy density. The constant B is a universal energy density associated with quarks⁵; the observed properties of hadrons suggest that B is $\sim 10^{35}$ erg cm⁻³. From equation (1) one finds that the pressure of matter at superhigh densities is

$$P = \frac{A}{3}n^{4/3} - B \quad (2)$$

That the pressure given by equation (2) is negative when the baryon number density is $< (3B/A)^{3/4}$ shows⁴ that below a certain pressure quark matter will fragment into hadrons. From equations (1) and (2) one finds that $P = \rho/3$ when $\rho \gg 4B$. Thus the speed of sound in matter $(dP/d\rho)^{1/2}$ asymptotically approaches $c/\sqrt{3}$ at superhigh densities.

That matter at superhigh densities can be described as a gas of quasi-free quarks is of course a highly useful piece of information when one wishes to discuss those astrophysical situations where superdense matter occurs. For example, knowing the behaviour of matter at superhigh densities allows extrapolation of the behaviour of an expanding universe back to very early times. In the 'big bang' model⁶ of the Universe, energy densities $\gg 4B$ will occur at times $t < 10^{-5}$ s, and thus the free quark picture should be valid at those times. In the following I will show that this has important implications for the question of the origin of the entropy of the Universe and whether primordial black holes exist.

The entropy of the Universe

The good agreement between the observed abundance of helium and the abundance of helium theoretically expected⁷ from hot big bang nucleosynthesis suggests that the Universe was already hot enough to be radiation dominated when the Universe was 1 s old. Further, the agreement between calculations⁸ of the amounts of deuterium, ³He, and ⁷Li formed during the time of helium synthesis and the observed abundances of these isotopes is strong evidence that the entropy of the Universe at 1 s was approximately equal to its present value, which is essentially just that contained in the cosmic microwave background and is, in Boltzmann's constant units, $\sim 10^9$ per baryon. Why the Universe has such a large entropy per baryon is unclear.

Ya. B. Zel'dovich has suggested⁹ that the entropy results from the dissipation of sound waves during very early epochs. These sound waves could be the result of small scale primordial density fluctuations. The existence of primordial density fluctuations on very large mass scales may explain the origin of galaxies. Zel'dovich points out that if there were the same level of fluctuations that gave rise to galaxies present on very small mass scales, then the observed entropy per baryon can be explained provided the equation of state of matter at superhigh densities approaches the form $P = \rho c^2$. The assumption that the pressure approaches ρc^2 at superhigh densities is crucial because it leads to very large internal energies per baryon. One can show that if $P = \rho c^2$ then the energy per baryon $\propto n$. Although such an asymptotic behaviour is consistent with

phenomenological nucleon–nucleon potentials¹⁰, it is not consistent with the free quark picture.

Asymptotic freedom and entropy

The energy density associated with a sound wave is

$$u_s = \varepsilon^2 \rho c_s^2 \quad (3)$$

where $\varepsilon = \delta\rho/\rho$ measures the maximum deviation from uniform density and c_s is the speed of sound. If the acoustic energy density u_s is completely dissipated then an equivalent amount of heat energy will appear. Some of this heat energy will appear in the form of radiation (gluons and photons), and if this radiation is in thermal equilibrium then the entropy per baryon S will be

$$S \simeq \rho_r^{3/4}/n \quad (4)$$

where ρ_r is the radiation energy density. If $\rho = (n/M)^2$ then one finds from equations (3) and (4) that the radiation entropy per baryon will be large when $n \gg (M/\varepsilon)^3$. If $M \sim 1$ GeV this corresponds to densities $\gtrsim \varepsilon^{-3} \times 10^{17}$ g cm⁻³. On the other hand, if asymptotic freedom (equation (2)) applies at superhigh densities then one finds that the radiation entropy per baryon resulting from dissipation of a sound wave can never be larger than $(\varepsilon/3)^{3/2}$. This quantity can never be large in an approximately homogeneous Universe where $\varepsilon \ll 1$.

Even though the entropy per baryon resulting from dissipation of a single sound wave is small, one might still ask whether dissipation of sound waves over cosmological time scales can lead to an appreciable buildup of entropy. Suppose, for example, that the dissipation of acoustic energy were fairly efficient, so that the energy density u_s at time t is dissipated in a time t . Then the radiation energy density ρ_r will evolve with time according to

$$\frac{d\rho_r}{dt} \simeq \varepsilon^2 \frac{\rho_m}{t} - 2 \frac{\rho_r}{t} \quad (5)$$

where ρ_m is the density of matter. The second term on the right hand side of this equation represents the effect of the expansion of the Universe on the radiation energy density. If we assume $\rho_r = 0$ at some initial time t_0 , then equation (5) together with the equations of general relativity imply that

$$\frac{\rho_r}{\rho_m} = \left[\frac{t}{t_0} \right]^{\varepsilon^2} - 1 \quad (6)$$

We see that $\rho_r \gg \rho_m$ only when $\ln(t/t_0)$ is large compared to ε^{-2} . It is not entirely clear what one should choose for t_0 , but the quantum theory of gravity seems to imply¹¹ that t_0 should be identified with the Planck time, $t_p = (\hbar G/c^5)^{1/2} \simeq 10^{-43}$ s. If this is the case an appreciable entropy could not have been generated by the time $t = 1$ s unless $\varepsilon \gtrsim 0.4$.

As to whether $\varepsilon > 0.4$ will in fact lead to a large entropy per baryon one must ask whether it is possible to have efficient dissipation over long periods of time. Actually, if ε is close to unity there will be some dissipation just from the fact that in such a situation black holes would be prolifically formed¹², and these black holes emit thermal radiation¹³. This radiation causes small black holes of mass M g to disappear completely in a time $\sim 10^{-27} M^3$ s (ref. 14). If we assume that black holes formed at time t have masses typically of the order of the Hubble mass $M_H = (4\pi/3) \rho (ct)^3$, then production of a large entropy per baryon by the time $t = 1$ s would require prolific production of black holes at times earlier than 10^{-28} s. It can be shown that the entropy per baryon generated by the decay

of a black hole formed at time t is $\simeq (t/t_p)^{1/2}$. Thus at least 99% of the baryons initially present would have to be processed through black holes to produce the observed entropy of $\sim 10^9$ per baryon.

An anisotropic Universe?

By choosing a value for ε close to unity for times $t < 10^{-28}$ s one can apparently produce a very large entropy per baryon by the time the Universe is 1 s old. The main difficulty with this explanation is that in order not to conflict with the observed large scale homogeneity of the Universe and lack of a large present-day density of primordial black holes¹⁵, the value of ε must somehow decrease from being close to unity for very small mass scales to being rather small ($\lesssim 0.01$) on very large mass scales. We do not understand how or why ε should vary with mass scale. One possible way out of this difficulty might be to suppose that the Universe is actually rather homogeneous on all mass scales but in addition is anisotropic¹⁶. It has been shown¹⁷ that if weakly interacting particles are present in such a Universe then the pattern of anisotropic expansion can be altered. In particular, if the mean free time between collisions is comparable with the expansion time, then strong damping of the anisotropy can occur¹⁸. The mean time for a large angle quark–quark scattering is

$$\tau \simeq \frac{1}{\pi g^2} \frac{E_q^2}{n_q c} \quad (7)$$

where E_q is the quark energy and n_q is the quark density. Since $E_q \propto V^{-1/3}$ in a collision dominated Universe we have $\tau \propto V^{1/3}$ as long as $\tau \ll t$. The expansion time is, however, proportional to V in an anisotropic Universe. Thus one will always have an initial stage of the Universe where τ is longer than the expansion time. During this initial period when the quarks are completely free, the quarks moving in certain spatial directions will be 'blueshifted' in energy rather than redshifted as in a collision-dominated Universe, and one will build up counter-streaming beams of energetic quarks along certain spatial directions. Because these beams are unstable from the quark plasma analogue of the two-stream instability, the buildup of these beams will ultimately result in entropy generation. One can calculate the rate of entropy production from the theory of two-stream instabilities in relativistic plasmas; however, this mechanism of entropy production lacks appeal at the present time as an explanation of the observed entropy because the duration of the anisotropic period and hence the entropy produced is a free parameter that can only be determined from the observed value of the entropy.

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Structural patterns in globular proteins

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A simple diagrammatic representation has been used to show the arrangement of α helices and β sheets in 31 globular proteins, which are classified into four clearly separated classes. The observed arrangements are significantly non-random in that pieces of secondary structure adjacent in sequence along the polypeptide chain are also often in contact in three dimensions.

ONE of the central problems in molecular biology is the formation of the native structure of a protein from the newly synthesised unfolded "structureless" polypeptide chain. In suitable conditions this process occurs spontaneously and the final conformation is determined solely by the amino acid sequence. As the random search of all possible conformations of the whole molecule would take an impossibly long time, this process probably involves intermediate structures that allow the protein to find its native conformation rapidly. Considerable experimental and theoretical effort has been devoted to trying to establish the nature of these intermediates.

In this article we first use a simple two-dimensional representation to illustrate the known conformations of 31 proteins. After classifying these known protein structures into four classes, we show that there is a strong tendency for pieces of secondary structure that are close together along the sequence also to be in close contact in the final three-dimensional structure. Such locally ordered regions, which are referred to here as folding units, associate to form the whole protein molecule, or in the case of some of the larger proteins, to form domains.

Diagrammatic representation of protein structure

Protein conformations are very complicated: it is not easy to comprehend the three-dimensional structure of a single protein, let alone compare many such structures. We have chosen to use a schematic two-dimensional representation similar to that used by certain other workers^{1,2}, and referred to here as topology/packing diagrams. The following rules were used in preparing our topology/packing diagrams for each protein. First, the α -helical and β -sheet chain segments are identified. As a β sheet can be formed from pieces of chain that are distant along the sequence, we use ' β ' strand to refer to a single piece of chain that forms one strand of a β sheet. Second, a viewing direction is defined so that most segments of secondary structure are viewed end on. Third, the protein is rotated about the line

of sight until the β strands lie in a horizontal plane (the twist of the β sheet is removed by flattening the sheet), and the front end of each β strand is drawn as a rectangle in the diagram. (This step is omitted if there is no β sheet.) Fourth, a circle representing the front end of each α helix is drawn, taking care to put segments that are close in space close together in the diagram. Finally, the segments are connected by bold or thin arrows (from the N to C terminal) that indicate whether the connection is at the near or far end, respectively, of the α helix or β strand. The scale of the real protein is preserved by making the separation of interacting α helices 10 Å, of hydrogen-bonded β strands 5 Å, and of other interacting β strands 10 Å. The diameter of the α -helix circle is 5 Å, and the β -strand rectangle is 4×5 Å.

Not all known protein conformations are included in the topology/packing diagrams given here (Figs 1–4). In some cases a particular structure is omitted as it is closely related to a protein that is shown: we show only one example of the immunoglobulin family^{3–11}, the trypsin family^{41,42} and the haemoglobin family^{43,44}. For a few proteins we could not find sufficiently clear pictures in the literature to be able to produce the diagrams (high potential iron protein⁴⁵, cytochrome c_2 (ref. 46), the catalytic part of alcohol dehydrogenase³⁰, malate dehydrogenase⁴⁷, ferredoxin⁴⁸, rhodanase⁴⁹, carbonic anhydrase⁵⁰ and soya bean trypsin inhibitor⁵¹). As these omitted proteins seem quite normal and fall into the present classification their omission should not affect our study.

Four clearly defined classes

The topology/packing diagrams of the 31 proteins are arranged into four classes defined as follows: (I) all- α proteins have only α -helix secondary structure (Fig. 1); (II) all- β proteins have mainly β -sheet secondary structure (Fig. 2); (III) $\alpha + \beta$ proteins have α -helix and β -strand secondary structure segments that do not mix but tend to segregate along the polypeptide chain (Fig. 3), and (IV) α/β proteins have mixed or approximately alternating segments of α -helical and β -strand secondary structure (Fig. 4).

Class I proteins are built up from α helices: more than 60% of the residues adopt the helical conformation. Because strongly interacting α helices are not always parallel (especially if the helices are short), the topology/packing diagrams do not show the packing of helices very accurately (Fig. 1). Of the three α -helical proteins shown, myohaemerythrin is most accurately represented as all the helices are parallel to the line of sight. The same packing of almost parallel α helices has

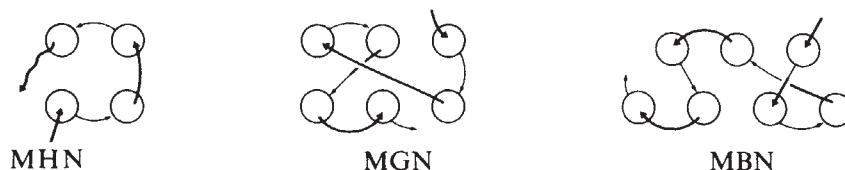
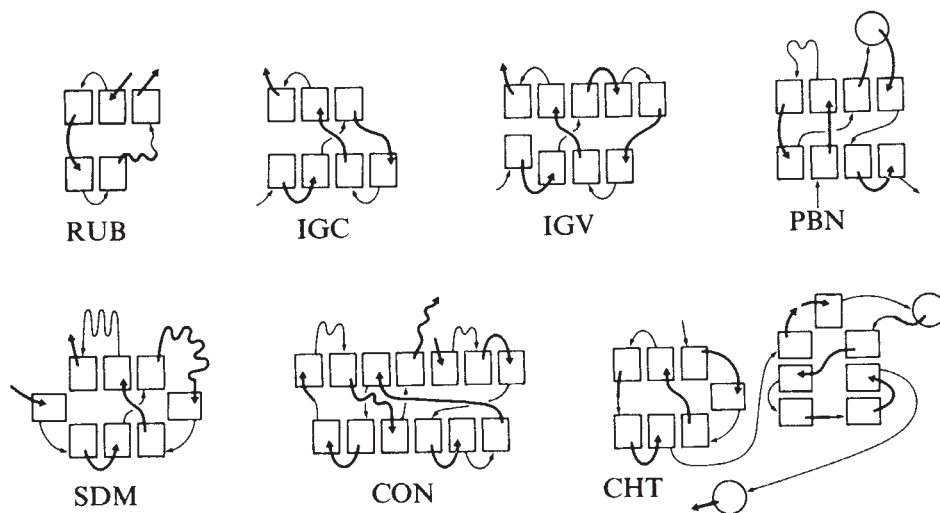


Fig. 1 Topology/packing diagrams of the following three all- α proteins (three-letter abbreviations have been assigned to all the proteins presented here): myohaemerythrin (MHN)³; myogen (MGN)⁴, and myoglobin (MBN)^{5,6}. As the axes of adjacent α helices are not always parallel and are sometimes at right angles, the diagrams cannot show the three-dimensional arrangement of helices very well.

Fig. 2 Topology/packing diagrams of the following seven all- β proteins in order of increasing size: rubredoxin (RUB)⁷; immunoglobulin constant region (IGC)^{8,9}; immunoglobulin variable region (IGV)^{10,11}; prealbumin (PBN)¹²; superoxide dismutase (SDM)¹³; concanavalin A (CON)^{14,15}; and chymotrypsin (CHT)^{16,17}. If the rectangles representing two β strands are very close together then the strands are hydrogen-bonded together. In some cases the associations are less clear in the diagrams: the final β strand of IGV also hydrogen bonds to the first strand; the first strand of SDM hydrogen bonds to both the second and final strands, and the fifth strand hydrogen bonds to both the fourth and sixth strands; in each domain of CHT, the second strand hydrogen bonds to both the first and third strands. The approximate twofold symmetry relationship between the upper and lower β sheets of the immunoglobulin regions and superoxide dismutase is clear (the twofold axis lies horizontally between the sheets).



been found in low resolution X-ray studies of tobacco mosaic virus coat protein^{52,53} and electron microscopy of purple membrane protein⁵⁴.

Class II proteins (Fig. 2) are built from β sheets stacked to form a layered structure. Although the number of β strands in each sheet varies from two (in rubredoxin) to seven (in concanavalin A), there are always two layers of β sheet. Adjacent β strands in these proteins run in opposite directions to form antiparallel sheets. All these class II proteins, except chymotrypsin, have only one domain, and in chymotrypsin the two domains have identical topological connections. Often the strands that occur near the ends of the polypeptide chain are positioned in the middle of the β sheet so that they are well stabilised with hydrogen-bond associations to two neighbouring β strands. In three cases, the β strand at the edge of one β sheet

also hydrogen bonds to the other β sheet closing one edge of the double layer (in the immunoglobulin variable region, superoxide dismutase and chymotrypsin), and in one of these (superoxide dismutase) both ends of the double layer are closed to form a barrel of β strands. The close similarity of the chain fold in the immunoglobulin variable region and superoxide dismutase, which has been pointed out before, is clear in Fig. 2.

Class III, the $\alpha + \beta$ proteins (Fig. 3), consist of a mixture of all- α and all- β regions within the same polypeptide chain. Often there is a cluster of helices at one or both ends of the β sheet, which is almost always built up from antiparallel strands. Only the three largest $\alpha + \beta$ proteins can be split into two relatively stable domains, and in each case one domain is mainly α helical while the other is mainly β sheet (T4 lysozyme, papain and thermolysin).

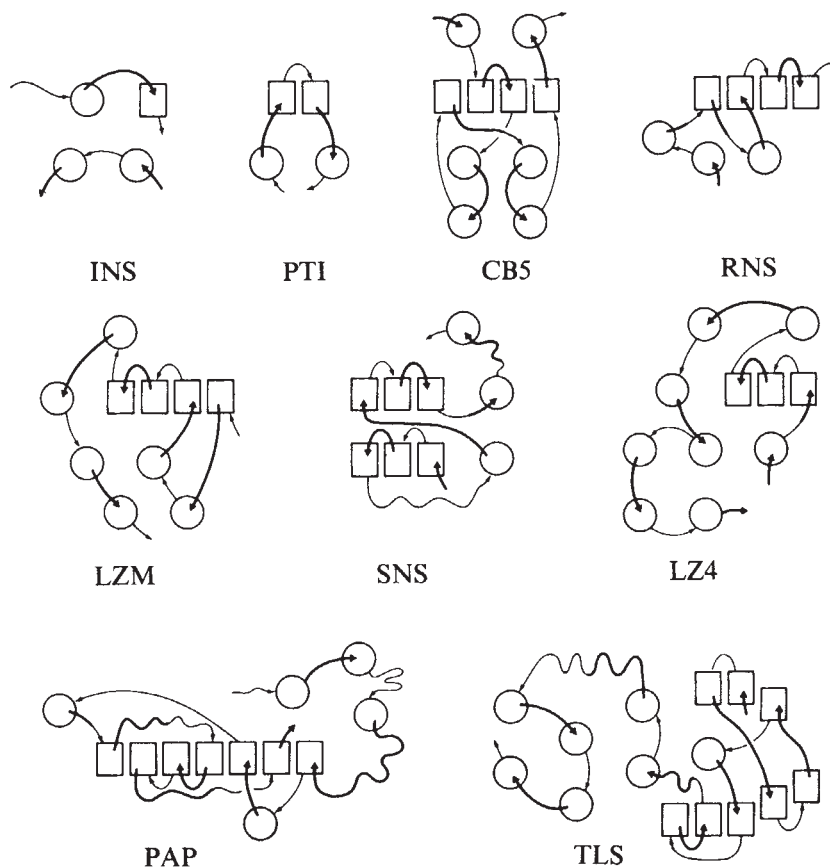


Fig. 3 Topology/packing diagrams for ten $\alpha + \beta$ proteins in order of increasing size: insulin (INS)¹⁸; pancreatic trypsin inhibitor (PTI)¹⁹; cytochrome *b₅* (CB5)²⁰; ribonuclease (RNS)²¹; hen lysozyme (LYM)²²; staphylococcal nuclease (SNS)²³; T4 lysozyme (LZ4)²⁴; papain (PAP)²⁵; and thermolysin (TLS)²⁶. The diagrams show that the two lysozyme structures are more similar than thought previously²⁴; analogy with the mammalian enzyme supports the idea²⁴ that the active residues of T4 lysozyme must be a glutamic acid at the end of the α helix preceding the β sheet and an aspartic acid at a bend in the β sheet (Glu 11 and Asp 20, respectively).

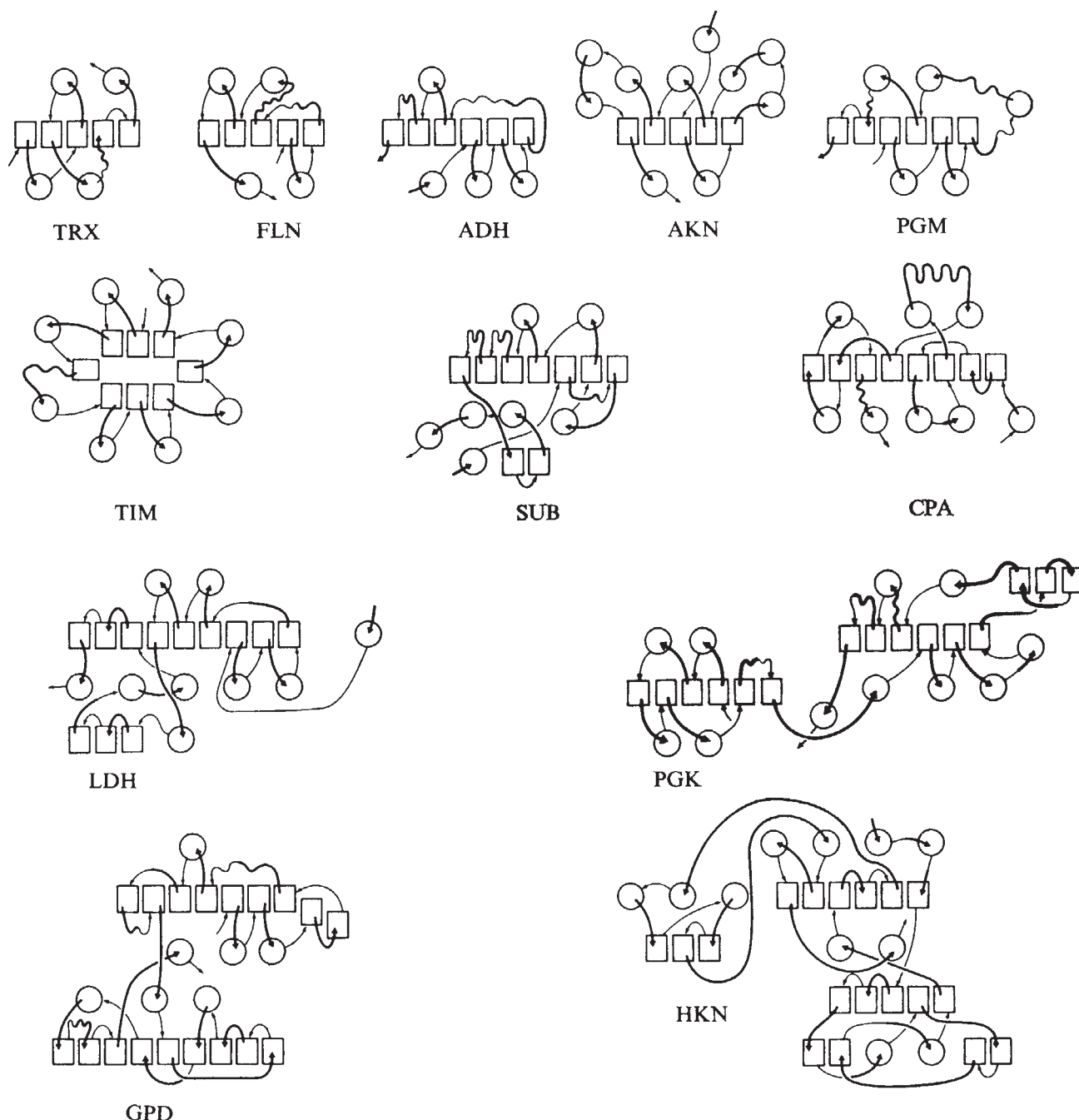
Class IV proteins, the α/β proteins (Fig. 4), have α helices and β strands that occur one after the other so that most α helices are separated by β strands along the sequence and vice versa. Most of these proteins have a single sheet surrounded by α helices, but in some of the larger proteins there are extra, smaller β sheets. The α helices pack on both sides of the β sheet, with α helices that follow one another along the chain, often on the same side of the β sheet. The main β sheet of each protein has between five and nine strands and consists mainly of parallel strands. The extra β sheets are smaller, often antiparallel, and more like the β sheets of the $\alpha + \beta$ proteins in class III (SUB and LDH in Fig. 4). Only the five largest of these α/β proteins (more than 300 residues) can be separated clearly into two or three domains. A common feature of these proteins

is the order of the β strands in the β sheet: in most cases the first strand is in the middle of the sheet, with the following strands along the chain added first to the right and then to the left of the first strand. Because most of the α/β proteins shown here are enzymes in the glycolytic pathway and have to bind a common coenzyme, NAD, some of the common features in Fig. 4 may be a result of these proteins having evolved from a common ancestor².

General features of known proteins

The interior hydrophobic core so characteristic of globular proteins is generally formed by contacts between and among α helices and β strands. The pieces of chain (often quite short) that connect these regions of secondary structure are generally

Fig. 4 Topology/packing diagrams of 12 α/β proteins in order of increasing size: thioredoxin (TRX)²⁷; flavodoxin (FLN)^{28,29}; alcohol dehydrogenase coenzyme domain (ADH)³⁰; adenyl kinase (AKN)³²; phosphoglycerate mutase (PGM)³¹; triose phosphate isomerase (TIM)³³; subtilisin (SUB)³⁴; carboxypeptidase (CPA)³⁵; lactate dehydrogenase (LDH)³⁶; phosphoglycerate kinase (PGK)^{37,38}; D-glyceraldehyde-3-phosphate dehydrogenase (GPD)³⁹; and hexokinase (HKN)⁴⁰. Because the upper and lower domains of GPD and HKN are not directly under the upper domains, the interactions are not as strong as implied by the figures. The arrangement of the lower domain of HKN must be regarded as only tentative as it was determined from a mono drawing of this very large protein⁴⁰.



exposed to solvent and contain most of the hydrophilic and bend-promoting residues. Most proteins can be considered crudely as a layered sandwich structure, with each layer consisting entirely of either α helices or a β sheet. In the all- α proteins or all- β proteins, there are only two such layers. In the $\alpha+\beta$ proteins there are also often two layers, and often one layer consists of α helices and the other is a β sheet, though more mixed arrangements occur. In the α/β proteins, there are three layers, with a layer of α helices on each side of the central β sheet. Triose phosphate isomerase may seem an exception in that there are four layers with a central β -sheet sandwich surrounded on both sides by α helices, but this protein can also be considered as a layer of β sheet and a layer of α helices rolled into a cylinder so that every β strand hydrogen bonds to two others. Although β strands are sometimes surrounded on all sides by other segments of secondary structure, α helices are almost always only partially buried. It is also rare to find an α helix that is not in contact with at least one other α helix.

Very often several secondary structure segments that are adjacent along the polypeptide chain also interact strongly in three dimensions to form what is defined here as a 'folding unit'. There are 12 possible combinations of α helices and β strands into sequences of two or three adjacent segments of secondary structure: ($\alpha\alpha$), ($\alpha\beta$), ($\beta\alpha$), ($\beta\beta$), ($\alpha\alpha\alpha$), ($\alpha\alpha\beta$), ($\alpha\beta\alpha$), ($\beta\alpha\alpha$), ($\alpha\beta\beta$), ($\beta\alpha\beta$), ($\beta\beta\alpha$) and ($\beta\beta\beta$). Not all these secondary structure segments interact to form a stable complex. Those combinations containing a single β strand must be excluded as that strand can neither interact strongly with an α helix nor hydrogen bond to another β strand in the folding unit to form a β sheet. The most important folding units, therefore, consist of the following groups of adjacent secondary structure segments: ($\alpha\alpha$), ($\beta\beta$), ($\beta\beta\beta$) and ($\beta\alpha\beta$). The ($\alpha\alpha$) folding unit consists of a pair of α helices adjacent in sequence that are arranged with their axes approximately antiparallel and are in van der Waals' contact (Fig. 5a). The ($\beta\beta$) folding unit consists of two β strands that fold back and hydrogen bond together into a β sheet with two antiparallel strands (Fig. 5b). The ($\beta\beta\beta$) folding unit is just an extension of the ($\beta\beta$) folding unit with an extra β strand forming a β sheet with three antiparallel strands arranged in a simple zigzag. The ($\beta\alpha\beta$) folding unit consists of a β sheet with two parallel strands in van der Waals' contact with an α helix antiparallel to these strands (Fig. 5c). Three other possible groups, ($\alpha\alpha\alpha$), ($\alpha\beta\beta$) and ($\beta\beta\alpha$), are rare in globular proteins, difficult to identify unambiguously and can be considered as formed from ($\alpha\alpha$) or ($\beta\beta$) folding units.

Table 1 gives the number of times the different folding units occur in all the globular proteins considered here. For some of the α/β proteins (class IV) adjacent ($\beta\alpha\beta$) folding units have a β strand in common. As folding units are defined here as independent subassemblies of secondary structure, a particular segment of secondary structure should not be part of two folding units. A new type of folding unit, referred to as ($\beta\alpha\beta$)', was introduced to take care of the case ($\beta\alpha\beta\alpha\beta$), that is, where a ($\beta\alpha\beta$) folding unit shares a β strand with an adjacent ($\beta\alpha\beta$) unit. For example, triose phosphate isomerase is formed from eight ($\beta\alpha\beta$)', folding units.

In the three all- α proteins there are eight ($\alpha\alpha$) folding units. If every α helix in these three proteins was in contact with the adjacent helices along the sequence, the maximum possible number of ($\alpha\alpha$) folding units would also be eight. In the seven all- β proteins there are 20 ($\beta\beta$) and four ($\beta\beta\beta$) folding units, respectively; the maximum numbers that could be formed are 24 ($\beta\beta$) and six ($\beta\beta\beta$). In the nine $\alpha+\beta$ proteins, there are 12 ($\alpha\alpha$), five ($\beta\beta$) and six ($\beta\beta\beta$) folding units, respectively; the maximum numbers that could occur for these proteins are 16 ($\alpha\alpha$), seven ($\beta\beta$) and six ($\beta\beta\beta$). In the twelve α/β proteins there are six ($\alpha\alpha$), 10 ($\beta\beta$), four ($\beta\beta\beta$), 18 ($\beta\alpha\beta$) and 12 ($\beta\alpha\beta$)', folding units, respectively; the maximum possible numbers are 12 ($\alpha\alpha$), 12 ($\beta\beta$), four ($\beta\beta\beta$), 23 ($\beta\alpha\beta$) and 15 ($\beta\alpha\beta$)'.

The above results show that for each class of protein the actual number of folding units observed is close to the maximum

possible number. For all the 31 proteins together there are 26 ($\alpha\alpha$), 35 ($\beta\beta$), 14 ($\beta\beta\beta$), 18 ($\beta\alpha\beta$) and 12 ($\beta\alpha\beta$)' folding units, respectively. A total of 242 segments of secondary structure belong to one or other of these folding units out of a total of 361 segments (67%). It is interesting that in the all- β proteins more β strands are in ($\beta\beta$) rather than ($\beta\beta\beta$) folding units, whereas in the $\alpha+\beta$ proteins more β strands occur in ($\beta\beta\beta$) rather than ($\beta\beta$), folding units.

Rao and Rossmann⁵⁵ concluded from an examination of four proteins that a structure consisting of three parallel β strands and two joining α helices was a common structural building block in proteins. (This structure consists of two overlapping ($\beta\alpha\beta$)' folding units.) They also noticed that the structure had a unique hand, in that the polypeptide chain is directed from β to α to β in a clockwise sense (Figs 4 and 5c). Sternberg and Thornton⁵⁶ have confirmed this handedness from a study of many protein structures and concluded that it arises from the twist of the β sheet. In another analysis of β sheets in protein structures, Richardson *et al.*⁵⁷ also noticed the high frequency of ($\beta\beta$) and ($\beta\alpha\beta$) folding units.

Statistical significance of patterns

Another way to assess the statistical significance of the arrangement of secondary structure in known proteins is to estimate the probability that a particular observed pattern would occur by chance. As the topology/packing diagrams represent the arrangement in space of α helices less well than of β strands, we first considered the statistical significance of the connectivity of the strands in the β sheets of proteins in classes II to IV. A computer program was used to generate random permutations of the order of the different β strands along the sequence while preserving the relative positions of the strands in the β sheets of the particular protein (the direction of the chain in the segments was not taken into account). For each of the 1,000 permutations generated for a particular protein, we counted the number of times a pair of β strands adjacent in the permuted sequence were also in contact in the β sheet (referred to as the number of adjacent contacts). This same

Fig. 5 The folding of the polypeptide chain in the three commonly occurring folding units: ($\alpha\alpha$), ($\beta\beta$) and ($\beta\alpha\beta$). The ribbon illustrates the path of the backbone with the arrows directed from the N to C terminals. Below each drawing of the chain fold is shown the representation of the particular folding unit used in the topology/packing diagrams of Figs 1-4.

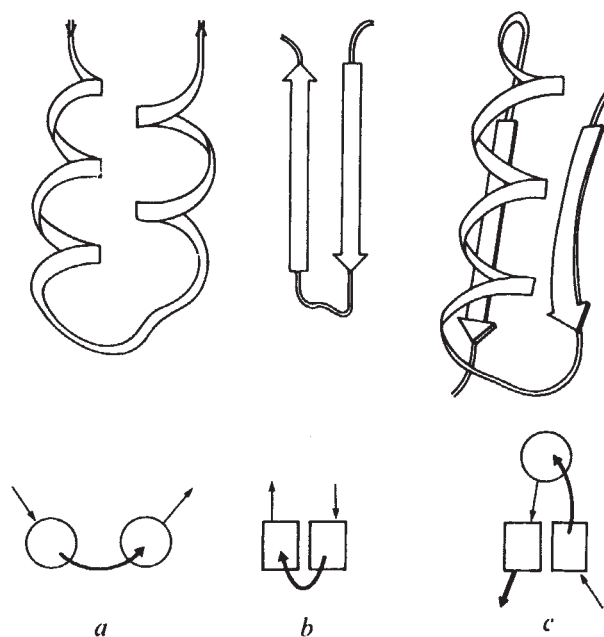


Table 1 Folding unit counts, β -sheet patterns, β -strand polarity and chance probability of 31 proteins

Table 1 Folding unit counts, β -sheet patterns, β -strand polarity, and strand polarity									
Class and protein		No. of folding units				β -Sheet pattern†	Strand polarity*		Probability
	($\alpha\alpha$)	($\beta\beta$)	($\beta\beta\beta$)	($\beta\alpha$)	($\beta\alpha\beta$)'		Np	Nap	
All- α									
Myohaemerythrin	2								0.33
Myogen	3								0.42
Myoglobin	3								0.14
All- β									
Rubredoxin		2				+- +-		3	0.37
Ig constant		3				+- -+-+		5	0.14
Ig variable		3	1			+- -+-+		7	0.006
Prealbumin		2				+- -+-+	1	5	0.16
Superoxide dismutase		3				+- -+-+		8	0.007
Concanavalin A		2	2			+- -+-+		11	0.001
Chymotrypsin		5	1			+- -+-+		12	0.05
$\alpha + \beta$									
Insulin	1								1.0
Trypsin inhibitor		1				+-		1	1.0
Cytochrome b5	2	1				- - + -	1	2	0.93
Ribonuclease	1		1			- + - +		3	0.08
Lysozyme (hen)	2		1			+ - + -		3	0.51
Staph.nuclease			2			+ - + + - +		4	0.006
Lysozyme (T4)	3		1			+ - +		2	0.33
Papain	1		1			- - + - + - +	1	5	0.54
Thermolysin	3	3				- + + - + + -	3	3	0.17
α/β									
Thioredoxin		1		1		- - - + -	2	2	0.56
Flavodoxin				2	1	- - - - -	4		0.15
Alcohol dehydrog.				2	1	- - - - -	5		0.21
Adenylate kinase	2					- - - - -	4		0.56
Phosphoglycerate mutase		1		1		- + - - - -	3	2	0.59
Triose phosphate isomerase					8	- - - - -	8		0.001
Subtilisin	1	1		2		- - - - - + -	6	1	0.24
Carboxypeptidase		1		1		+ + - - - - +	4	3	0.25
Lactate dehydrog.	1		2	2	2	- + - - - - - - + -	6	4	<0.001
Phosphoglycerate kinase	1			4		- - - - - + - + +	9	3	<0.001
Glyceraldehyde phosphate dehydrog.		3		3		- - - - - + + + - - - + - + - +	9	7	<0.001
Hexokinase	1	2	2	1		+ - + + - - - + - + - +	5	8	0.003
						+ - + - - -			

*The numbers of parallel β -strand associations in the β sheet(s) are given in the column headed Np, and the number of antiparallel associations are given in the column headed Nap.

†The β -sheet pattern is taken from Figs 2-4 with a plus sign to mark β strand that runs away from the viewer (into the paper), and a minus sign to mark a β strand that runs towards the viewer (out of the paper).

number was obtained for the native protein arrangement from Figs 1-4. As the α helices are ignored, β strands in contact in the sheet but separated in sequence by only one α helix were also counted as adjacent contacts. The statistical significance of the native arrangement was taken as the probability that a randomly generated permutation of strand order in the particular β sheet would have at least as many adjacent contacts as the native arrangement. These probabilities (P) are given in the last column of Table 1 and vary from 1.0 to 0.001 with a geometric mean of 0.05. For the smallest β sheet consisting of only two β strands, any permutation of the strands will leave them in contact in the sheet so the probability of having at least one adjacent contact is 1.0 (for example, trypsin inhibitor, Fig. 3). For a β sheet with three strands, there is a one-third chance of forming an antiparallel arrangement

with two adjacent contacts (for example, T4 lysozyme, Fig. 3). As the size of the β sheet increases the chances of having many such adjacent contacts decreases. For example, the β sheet in both cytochrome b_5 and ribonuclease consists of four strands (Fig. 3), but the arrangement in the former protein is almost random (one adjacent contact, $P = 0.33$), whereas that in the latter is highly significant (three adjacent contacts, $P = 0.08$). One of the three α/β proteins with a five-stranded β sheet (Fig. 4), flavodoxin has a more significant pattern (three adjacent contacts, $P = 0.15$) than the other two (both thioredoxin and adenylate kinase have two adjacent contacts each and $P = 0.56$). The patterns found in the proteins with the biggest β sheets are very significant, with P values less than 0.01, and in the case of some of the dehydrogenases, less than 0.001. These results show that β strands that are adjacent in

sequence are in van der Waals' or hydrogen-bonding contact much more often than expected by chance. Had the connectivity of the α helices also been considered, the observed patterns would be even more significant as then there would be many more segments of secondary structure to permute. The significance of the patterns of the all- α proteins was also estimated in this way but now the helices could be in contact both vertically and horizontally in the topology/packing diagrams. The results of this calculation (Table 1) show that none of these patterns of the all- α proteins is statistically very significant.

Parallel and antiparallel β sheets

Table 1 also shows the polarity of the β strands in the β sheets of the proteins considered here. For the all- β proteins (class II, Fig. 2), only one out of the 50 hydrogen-bonding associations in the β sheets is between a pair of chains that run in the same direction (parallel) rather than in opposite directions (antiparallel); it is in prealbumin. For the $\alpha+\beta$ proteins (class III, Fig. 2) most of the β strands also form antiparallel rather than parallel associations (23 and five occurrences, respectively). On the other hand, the β sheets of the α/β proteins (class IV, Fig. 4) have many more parallel associations than antiparallel ones (64 and 21 occurrences, respectively). When the β strands are grouped together along the sequence and not separated by α helices (as in the all- β and $\alpha+\beta$ proteins), ($\beta\beta$) and ($\beta\beta\beta$) folding units occur very often leading to antiparallel β sheets. When the β strands are separated by α helices along the sequence (as in the α/β proteins), ($\beta\alpha\beta$) and ($\beta\alpha\beta$)' folding units occur most often leading to parallel β sheets.

Conclusions and implications

In the past the word domain has been used to describe substructures in protein molecules. This use derives from the observation of Phillips⁵⁸ who described the structure of lysozyme in terms of a series of "compact globular units". The word domain has also been used to describe the independent globular regions that can occur when a single polypeptide chain is formed by gene fusion or gene duplication (for example, the four domains of the immunoglobulin heavy chains). The use of the same word "domain" to define both types of protein substructure leads to confusion, and we suggest that the phrase 'folding unit' be used to define small assemblies of secondary structure segments that are adjacent in sequence and in van der Waals' or hydrogen-bonding contact with one another. The word domain is then reserved for large subassemblies that would be stable if the polypeptide chain connecting them to the rest of the protein molecule were to be cleaved⁵⁹. Each domain has all the characteristics of a complete globular protein; often the different domains of a multidomain protein have different functions⁶⁰.

Our analysis of known protein structures using simplified topology/packing diagrams has shown that the observed arrangements of α helices and β strands are statistically very significant. The classification of 31 protein structures into four clearly separated classes has made it possible to identify common structural patterns, and has shown how the arrangement of segments of secondary structure along the sequence relates to three-dimensional properties such as parallel and antiparallel β sheets. Very often segments of secondary structure adjacent in sequence form a few well defined types of folding units. The high frequency of folding units in globular proteins is probably a result of the kinetic pathway of protein assembly rather than the stability of the final folded form. If certain segments of secondary structure existed with even marginal stability when the native conformation was unfolded, those segments that were close along the polypeptide chain would have a higher probability of diffusing together to form folding units⁶¹. If these folding units were then to associate rapidly to form the native conformation, which might be in a kinetically determined

minimum of the free energy, the high frequency of such units in native proteins would be expected. The idea that segments of secondary structure close in sequence interact to form subassemblies which then associate to form the native structure has been used by Ptitsyn and Rashin to study the folding pathway of myoglobin⁶². Wetlaufer⁶³ used a similar kinetic argument to explain the domain structure of proteins. Other explanations of the high frequency of folding units in globular proteins do not depend on kinetic arguments: for example, the native conformation could be stabilised if its segments of secondary structure were to be connected up by short pieces of chain that do not cross one another. The chance probability that two evolutionary unrelated proteins have similar arrangements of secondary structure will be less than thought previously¹, for we have shown that all protein conformations are built up from the same three basic folding units and that the conformations fall into four well defined classes.

Thus the picture of protein folding suggested by the final protein structure is as follows: pieces of secondary structure first diffuse together to form folding units that then associate to form the native structure, or in the case of the larger proteins, the domains which subsequently interact weakly to form the native structure.

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Identification of an enzyme in platelet microsomes which generates thromboxane A₂ from prostaglandin endoperoxides

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The microsomal fraction of horse and human platelets contains an enzyme which converts prostaglandin cyclic endoperoxides (PGG₂ or PGH₂) to a substance which is much more potent in contracting strips of rabbit aorta. This substance has the same characteristics as thromboxane A₂, and can be distinguished from other products of arachidonic acid metabolism by differential bioassay.

PIPER and Vane¹ have detected the release of a substance during anaphylaxis in isolated lungs from sensitised guinea pigs which they called "rabbit aorta-contracting substance" or RCS. The half life of RCS was <2 min at room temperature² and its release was blocked by aspirin-like drugs. RCS contracts all isolated vascular tissues tested from several species² and is released from lung tissue also by bradykinin, RCS-RF² SRS-C³, arachidonic acid^{3,4} and mechanical agitation or stimulation^{2,4}. RCS is also generated by mechanical stimulation of rabbit spleen slices⁵ and by incubation of arachidonic acid with rabbit spleen microsomes⁵. In addition, platelet aggregation is accompanied by the generation of an RCS^{6–10}. RCS release has also been demonstrated *in vivo*, either by challenging sensitised guinea pigs with antigen or by infusion of bradykinin⁴.

The release of RCS by the prostaglandin precursor, arachidonic acid, and the prevention of release by inhibition of prostaglandin biosynthesis led to the suggestion that RCS is an intermediate in prostaglandin biosynthesis^{3,5}. A cyclic endoperoxide of arachidonic acid was postulated as the unstable intermediate in the biosynthesis of prostaglandins^{11,12}. Endoperoxides have been generated and isolated after incubation of arachidonic acid with sheep vesicular glands^{13,14}. The prostaglandin endoperoxides (now most frequently designated PGG₂ and PGH₂; Fig. 1) contract rabbit aorta, gastrointestinal and tracheal smooth muscle¹⁵ and at low concentrations induce aggregation of platelets^{10,16}. Aggregation induced by collagen, thrombin, adrenaline and arachidonic acid is also accompanied by release of endoperoxides^{10,16,17}. The inhibitory effect of aspirin on the second phase of platelet aggregation¹⁸ could therefore be due to inhibition of biosynthesis of platelet endoperoxides.

PGG₂ and PGH₂ are unstable, but their half lives are longer (*t*_{1/2} ≈ 5 min) than that of RCS^{10,16}. Furthermore, quantitative analysis of the endoperoxide released from either guinea pig lung effluent or human platelets showed that only a minor part of the rabbit aorta-contracting activity was due to prostaglandin endoperoxides¹⁰.

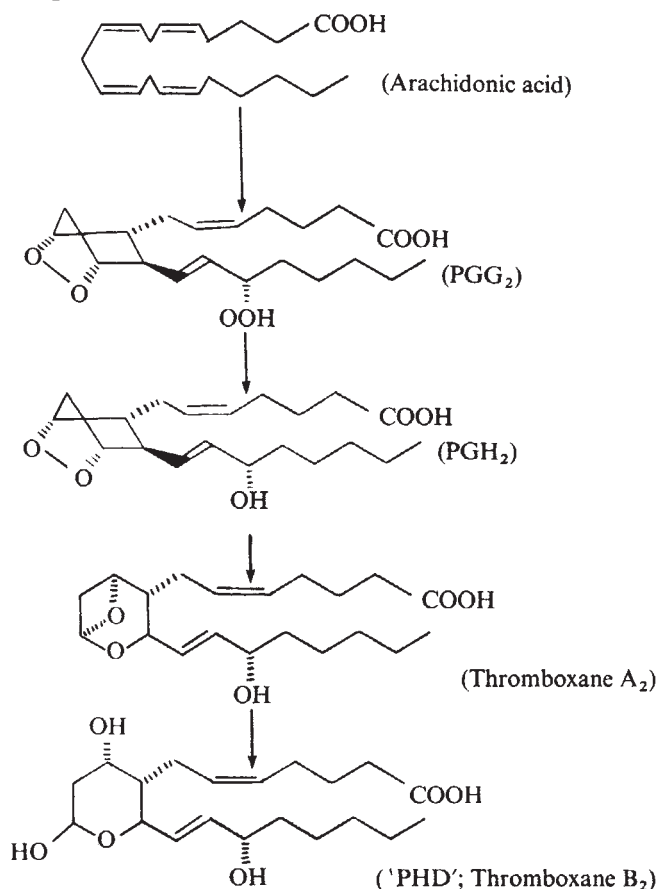
Samuelsson's group¹⁹ has discovered a labile intermediate in the conversion of the endoperoxide PGG₂ into the hemiacetal

derivative PHD (Fig. 1). They named the intermediate thromboxane A₂ (TXA₂) because of its structure and potent aggregating activity on platelets. This substance also strongly contracts rabbit aorta strips and its half life in aqueous solution is approximately 30 s. These properties of TXA₂ resemble very closely the properties of RCS, and it has been concluded that the activity of RCS is mainly due to TXA₂ (ref. 19).

Washed human platelets convert PGG₂ to TXA₂ (ref. 19). In the work reported here, we isolated from the same source and also from horse platelets an enzyme system which converts endoperoxides to a substance with the characteristics of TXA₂ and compared the relative contractile effects of endoperoxides and this substance on rabbit aorta.

Two litres of fresh citrated (3.8% w/v) blood from a horse were spun at 200g for 10 min to remove red blood cells. The

Fig. 1 Structures of some metabolites of arachidonic acid.



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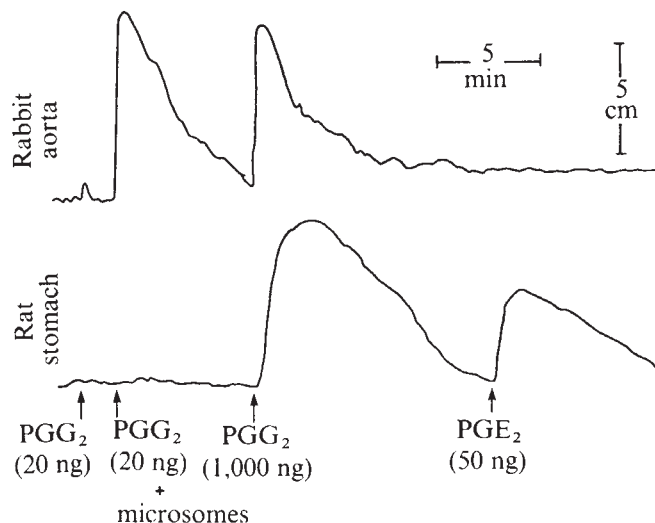


Fig. 2 Differential bioassay of rabbit aorta-contracting substances. The treatment conditions are indicated at the bottom of the figure. PGG₂ (200 ng) was added to 500 μ l of Tris buffer (50 mM, pH 7.8) at 0 °C and a 50- μ l sample (equivalent to 20 ng) was tested. Immediately after testing, horse platelet microsomes were added to the PGG₂ solution and 50 μ l was tested 2 min later.

platelet-rich plasma was centrifuged (2,000g) for 15 min and the pellet of platelets was resuspended in 20 ml of 100 mM Tris (pH 7.5) buffer. The platelet suspension was subjected to freezing and thawing three times. Disrupted platelets were centrifuged at 5,000g for 15 min to remove cell debris. The supernatant was spun at 100,000g for 60 min. The resultant pellet was washed and resuspended in 2 ml of 100 mM Tris (pH 7.5) buffer and homogenised.

Electron microscopy of the particulate fraction showed it to be composed of vesicles bound by single membranes 50–100 nm in diameter. Because of these characteristics, we refer to this fraction as microsomes. The protein content of the suspension used was 14.2 mg ml⁻¹, as determined by the method of Gornall *et al.*²⁰. A similar procedure was adopted in one experiment in which a particulate fraction from 1 litre of fresh human blood was prepared.

Biological activity was assayed on a rat stomach strip²¹ and a rabbit aorta¹ superfused at a rate of 10 ml min⁻¹ with Krebs solution at 37 °C, containing a mixture of antagonists²², to inactivate acetylcholine, 5-hydroxytryptamine, catecholamines and histamine, and also indomethacin (1 μ g ml⁻¹) to inhibit the synthesis of prostaglandins by the assay tissues²³.

Application of PGG₂ or PGH₂ caused the assay tissues to contract in a dose-dependent manner. When PGG₂ was incubated with horse or human platelet microsomes and tested, there was a much greater contraction of rabbit aorta than was caused by PGG₂ alone (Fig. 2). Indomethacin (up to 25 μ g ml⁻¹) did not affect the generation of the more potent compound. Similarly when PGG₂ was incubated with intact washed platelets, a powerful rabbit aorta-contracting substance (presumably TXA₂) was produced. But neither boiled platelet microsomes nor the 100,000g supernatant fraction of lysed platelets converted PGG₂ to the more potent compound. Furthermore, when PGG₂ had stood in aqueous solution at room temperature for 25 min, it no longer caused contraction of the aorta, nor did it yield an aorta-contracting substance when subsequently incubated with platelet microsomes. The substance generated from PGG₂ by platelet microsomes was highly unstable, with a half life at 37 °C of ~ 30 s. From these characteristics we assumed that the compound generated was TXA₂.

Figure 2 illustrates how the rabbit aorta and the rat stomach in a superfusion cascade can be used to distinguish between PGG₂, TXA₂ and PGE₂. PGG₂ (1,000 ng) caused both rabbit

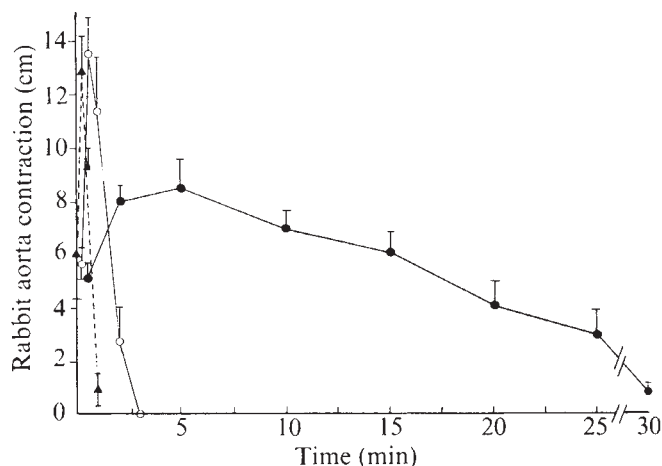


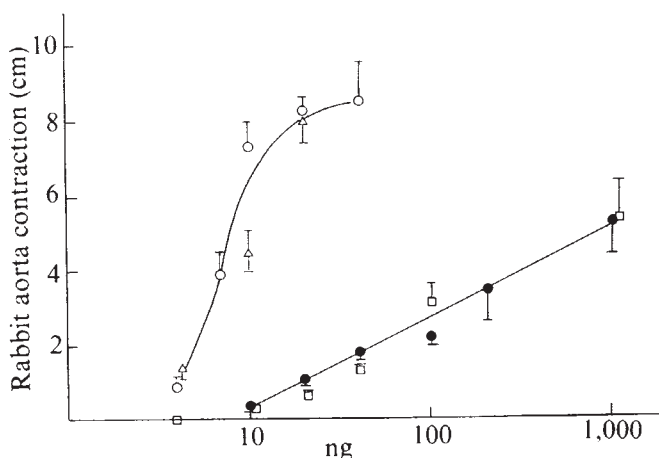
Fig. 3 Time course and temperature dependence of TXA₂ synthetase. PGG₂ (500 ng) was added to 1 ml of Tris buffer (50 mM, pH 7.8) and incubated at 0 °C (●), 22 °C (○) or 37 °C (▲) before bioassay (50 μ l). The values are the means ($\pm$ s.e.) of four experiments at each temperature.

aorta and rat stomach to contract. A lower dose (20 ng) of PGG₂, that had little or no effect on these tissues before incubation with platelet microsomes, yielded sufficient TXA₂ to cause a larger contraction of the rabbit aorta than 1,000 ng of PGG₂—more than a 50-fold increase in potency. TXA₂, (unlike PGG₂) had no effect on rat stomach, however. On the other hand, PGE₂ (50 ng) produced a large contraction of rat stomach without affecting rabbit aorta. It was 3 to six times more active than PGG₂ on the stomach.

The lability of TXA₂ in aqueous solution was reduced by lowering the temperature of the reaction mixture: at 0 °C, incubation of PGG₂ with platelet microsomes gave a reaction which peaked at 2–5 min and contractile activity was observed for more than 20 min (Fig. 3). When the reaction was carried out at 37 °C, the peak occurred at 15 s and activity disappeared within 1 min (Fig. 3). At room temperature (22 °C) reactions also proceeded rapidly and activity appeared in about 2 min.

The high potency of TXA₂, together with its very limited stability especially at 37 °C, suggests that this substance is a local hormone. For example, in haemostasis the formation of a

Fig. 4 The comparative potency of PGG₂ and TXA₂. Horse platelet microsomes (284 μ g of protein) were incubated with various concentrations of PGG₂ (Δ) or PGH₂ ($\circ$) in 500 μ l of Tris buffer (50 mM, pH 7.8) for 2 min at 0 °C before testing (50 μ l). Alternatively, PGG₂ ($\square$) or PGH₂ ($\bullet$) was incubated alone (2 min at 0 °C) in 500 μ l of Tris buffer (50 mM, pH 7.8). Values are means $\pm$ s.e. ($n = 3$).



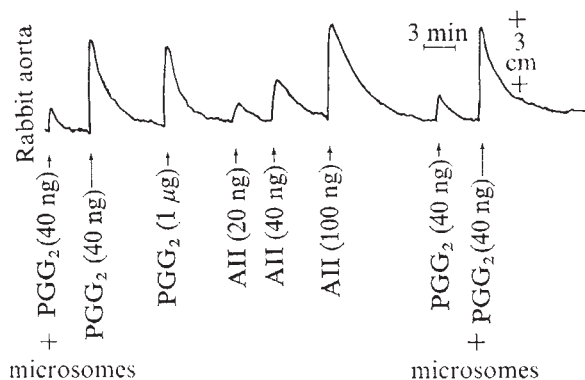


Fig. 5 Comparative potency of TXA₂ and angiotensin II (AII). Horse platelet microsomes (154 µg protein) were incubated with PGG₂ (200 ng) in 500 µl of Tris buffer (50 mM, pH 7.8) for 2 min at 0 °C before testing (100 µl equivalent to 40 ng PGG₂). Contractions to angiotensin II and PGG₂ alone are shown.

platelet plug should be facilitated when the lumen of the severed blood vessel is constricted. The endoperoxide and TXA₂ generated by aggregating platelets could produce a profound vasoconstriction largely restricted to the area of the vessel immediately adjacent to the platelet plug. Vasospasm associated with coronary or cerebral infarction could be produced by potent vasoconstrictors generated during platelet clumping.

Drugs that inhibit endoperoxide generation (for example, indomethacin) or inhibit TXA₂ biosynthesis could reduce vasoconstriction as well as platelet aggregation and thereby prolong bleeding time.

PGH₂ was also converted to a more potent aorta-contracting substance by platelet microsomes. A quantitative comparison of PGG₂ and PGH₂ and their activation by microsomes is presented in Fig. 4. The dose-response curves, however, are not parallel and it is difficult to assign a relative potency. As Figs 2 and 4 show, 1,000 ng of PGG₂ or PGH₂ applied directly to the aorta matched the contraction produced by 20 ng of PGG₂ (or PGH₂) when incubated for 2 min at 0 °C with platelet microsomes. This observation underestimates the potency of TXA₂ because (1) at 2 min the contractile activity represented the resultant of synthesis and destruction of TXA₂, and no correction for destruction was applied and (2) the comparisons were made on the basis of the degree of the contraction of the aorta but the response to TXA₂ usually lasted longer than that to endoperoxide.

Detection of low concentrations of endoperoxides could be amplified substantially by adding platelet microsomes to convert PGG₂ or PGH₂ to the more potent TXA₂. Similarly,

addition of platelet microsomes made it possible to discriminate between endoperoxides and TXA₂. For example, Willis²⁴ has described a "labile aggregation stimulating substance" (LASS) which aggregates platelets and is a potent aorta constrictor. If extracted LASS is an endoperoxide, platelet microsomes would be expected to enhance its contractile potency whereas the platelet enzyme would exert no effect on already formed TXA₂.

In a further experiment the activity of PGG₂ and TXA₂ in causing rabbit aorta to contract was compared with that of angiotensin II (Fig. 5). PGG₂ was less active (1/4–1/5th molar basis) than angiotensin II, whereas TXA₂ was at least as potent as angiotensin II. It is therefore conceivable that TXA₂, like angiotensin, will turn out to be a powerful vasoconstrictor in the body.

From these results we conclude that we have identified an enzyme in platelet microsomes which converts prostaglandin endoperoxides into the more potent and more unstable aorta constrictor, TXA₂. This has also been demonstrated directly by isolation of labelled thromboxane B₂ (Fig. 1) after incubation of ¹⁴C-arachidonate with a particulate fraction from human platelets (unpublished results of P. Falardeau and B.S.).

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letters to nature

Pulsar spin down and cosmologies with varying gravity

In this paper I show that some cosmologies with weakening gravity are in conflict with the measured spin down of the pulsar J1953. If gravity is weakening, it must do so even more slowly than the limits set by radar ranging. For group theory cosmologies with strengthening gravity, we also give a natural explanation for the lack of long period pulsars.

Certain very large dimensionless ratios of the fundamental constants of physics and astronomy exist. Dirac^{1,2}, in his large number hypothesis (LNH), has suggested that these ratios are interrelated and simple functions of cosmic time. Thus the gravitational constant G as measured by atomic standards varies with cosmic time as t^{-1} and the number of nucleons in the Universe varies as t^2 . Two possibilities for the implied continuous creation of matter were recently considered by Dirac^{3,4}: first, nucleons are created uniformly throughout space (additive creation) and second, that matter

is created where it already exists in proportion to the amount existing (multiplicative creation). The LNH can be used to generate four distinct cosmologies: the early Dirac cosmology^{1,2}, and the additive and multiplicative creation of matter cosmologies^{3,4}, and the cosmology of Hoyle and Narlikar⁵⁻⁷.

Recently a group theoretical analysis of isotropic and spatially homogeneous cosmological models has been presented by Malin⁸. This analysis strongly suggests that the mass of all particles varies inversely as the four-dimensional radius of curvature of the Universe. Some of the cosmological and astrophysical implications of this mass variation have been explored in papers by Malin⁸⁻¹⁰ and Mansfield and Malin¹¹. Although the genesis and implications of the G -changing and mass-changing cosmologies are quite different¹¹, here both types of theories are considered as just prescribing a time variation of the dimensionless gravitational coupling parameter $\gamma = Gm_p^2/\hbar c$. All other constants and coupling parameters remain constant, except in the case of additive and multiplicative creation, where the number of nucleons in the Universe, N , $\propto t^2$. In the G -changing cosmologies $\gamma \propto t^{-1}$ while a family of cosmological models based on the group analysis gives $\gamma(t_0)/\gamma(t_0) = 3H_0$. (I take H_0 , Hubble's constant¹² = 55 km s⁻¹ Mpc⁻¹).

For an arbitrarily relativistic, degenerate, zero temperature star, exact polytrope models can be constructed where the pressure and density are related by $p = K\rho^{(n+1)/n}$ where K and n are constant. For these polytropes¹³

$$\gamma N^{(n-1)/n} R^{(3-n)/n} = f_n \quad (1)$$

where N is the number of nucleons in the star, R its radius, and f_n is a function of n only which varies by 14% over the full range of stable polytropes. ($n = 1.5$ for non-relativistic stars and $n = 3$ for extreme relativistic stars.) All contributions from rotation, magnetic fields, nuclear forces, and relativistic gravity are neglected—a good approximation for my purposes.

The moment of inertia of a spherical star can be written $I = k m_e N R^2$ where for stable polytropic stars¹⁴ $0.04 \leq k \leq 0.132$. This definition and equation (1) can be combined to yield

$$\frac{\dot{I}}{I} = \frac{2n\dot{\gamma}}{(n-3)\gamma} + \frac{3n-5}{n-3} \frac{\dot{N}}{N} \quad (2)$$

where I neglect the very small variation of n and k with time.

For decreasing γ , I assume that the pulsar rotates as a solid body and that the main pulse period is the period of rotation. Then a firm lower limit to the spin down rate $\dot{P}/P$ can be found by setting all electromagnetic losses and thus external torques equal to zero. In the early cosmologies of Dirac^{1,2} and Hoyle and Narlikar^{5,7} this means that angular momentum is conserved and hence I/P is constant, whereas in the recent cosmologies of Dirac^{3,4} for an isolated star $I/P \propto MVR$ where the tangential rotation velocity, V , of the star is held constant rather than angular momentum. When angular momentum is conserved^{1,2,5-7} a maximum $P/\dot{P}$ is found for $n = 1.5$ (non-relativistic)

$$\left[\frac{P}{\dot{P}} \right]_{\max} = \left[\frac{I}{\dot{I}} \right]_{\max} = -0.5 \frac{\gamma}{\dot{\gamma}} \quad (3)$$

where I use the fact that in these cosmologies $\dot{N} = 0$.

When the velocity is held constant^{3,4} again the maximum is found in the non-relativistic extreme where

$$\left[\frac{P}{\dot{P}} \right]_{\max} = \left[\frac{R}{\dot{R}} \right]_{\max} = \begin{cases} \text{none (multiplicative creation)} \\ -\gamma/\dot{\gamma} \text{ (additive creation)} \end{cases} \quad (4)$$

This analysis does not provide any useful limit for $(P/\dot{P})_{\max}$ in

the case of multiplicative creation since then $\dot{P}/P$ can be positive, negative or zero (when $n = 2.0$) depending on the mass, and thus n , of the star. Equations (3) and (4) give instantaneous maxima—between 'glitches' for example.

The results of equations (3) and (4) are summarised in Table 1. From this it can be seen that the cosmologies of Dirac^{1,2} and Hoyle and Narlikar⁵⁻⁷ give, in the limit of zero electromagnetic losses, a $(P/\dot{P})_{\max} < [8.6 \pm 1.3(2\sigma)] \times 10^9$ yr, the measured value for JP1953 (ref. 15). In fact, for any cosmology that conserves angular momentum, equation (3) with JP1953 implies that when $\dot{\gamma} < 0$ then $-\dot{\gamma}/\gamma \leq [5.8 \pm 1(2\sigma)] \times 10^{-11}$ yr⁻¹ independent of H_0 . This restriction on decreasing γ is slightly better than the restrictions provided by radar ranging (I. I. Shapiro, personal communication).

The values in Table 1 are derived neglecting relativistic gravity. This can easily be justified by noting that the maximum mass that can be supported against gravity by a degenerate gas scales¹⁵ as $M_{\max} \propto \gamma^{-3/2} \propto t^{3/2}$. Thus old pulsars must be of very low mass and therefore relativistic gravity is unimportant. For a pulsar like JP1953, if $\dot{\gamma}/\gamma < 0$ its true age is actually greater than $0.5P/\dot{P} = 4.3 \times 10^9$ yr as predicted by the standard ($\gamma = \text{const.}$) spin down theory. Conservatively estimating its age at 4.3×10^9 yr, the maximum pulsar mass at its birth normalised by $M_{\max}(t_0)$ is seen to span the range

$$0.09 \leq M_{\max}(t_0 - 4.3 \times 10^9 \text{ yr})/M_{\max}(t_0) \leq 0.76$$

depending on which cosmology is considered. Thus it is clear that relativistic gravity is unimportant for old pulsars. In addition, the small effects of relativistic gravity would be overwhelmed by any reasonable spin down because of electromagnetic losses.

For increasing γ according to the group analysis $\dot{\gamma} > 0$, then $\dot{I} < 0$. This makes the pulsar spin up—counteracting the spinning down from electromagnetic losses. For either a dipole magnetic field inclined at an angle to the spin axis or for a standard pulsar magnetosphere model the loss rate $\simeq -B^2 R^6 \omega^4/c^3$, where B is the surface component of the dipole field at the polar cap¹⁶. Then the proper equation for the case when $\dot{I}(t)$ varies according to equation (2) with $\dot{N} = 0$ is

$$\frac{dP^2}{dt} = \frac{8\pi^2 B^2 R^6}{c^3 I} - \frac{4P^2 n \dot{\gamma}}{(3-n)\gamma} \quad (5)$$

Notice that $\dot{P} \rightarrow 0$ for a critical period given by

$$P_c = \left[\frac{2\pi^2 B^2 R^6 (3-n)}{3c^3 \ln H(t)} \right]^{1/2} \quad (6)$$

where $\dot{\gamma}/\gamma = 3H(t)$ as prescribed by the group theory analysis. P_c is the period at which the spin down from electromagnetic losses is just compensated for by the spin up because $\dot{I} < 0$. Of course, since $\dot{P} \rightarrow 0$ there is no limit for $(P/\dot{P})_{\max}$ in this cosmology.

$R^3/I^{1/2}$ can vary by almost an order of magnitude for the full range of stable neutron stars¹⁶. Models¹⁷ for the genesis of neutron stars suggest, however, that they should be formed with a mass near the Chandrasekhar limit $\sim 1.3M_\odot$. On the basis of these arguments Ruderman and Sutherland¹⁸ adopt a model in which $M = 1.25M_\odot$, $R = 8.1$ km, and $I = 6.24 \times 10^{44}$ gm cm². In these conditions $n \simeq 2$. Then equation (6) yields $P_c \simeq 5(B/10^{12} \text{ gauss})$. Thus for very reasonable pulsar parameters $P_c \simeq 5$ s with a factor of ~ 5 uncertainty because of the permissible range in pulsar parameters. Therefore when $\dot{\gamma}/\gamma = 3H_0$ as predicted by the group analysis, the pulsars should tend to a limiting period of $P_c \simeq 5$ s which is consistent with $P \leq 3.74$ s for the 147 known pulsars (Y. Terzian, personal communication). Thus, the increasing γ cosmology is consistent with the pulsar data and offers a natural explanation for the

Table 1 Maximum values of $P/\dot{P}$ for the decreasing γ cosmologies in the limit of zero electromagnetic losses. The measured $P/\dot{P} = [8.6 \pm 0.8(1\sigma)] \times 10^9$ yr for JP1953

Cosmology	t_0	$(P/\dot{P})_{\max}$	$(P/\dot{P})_{\max}$ (yr)
Early Dirac	$\frac{1}{2}H_0^{-1}$	$t_0/2$	3×10^9
Hoyle-Narlikar	$\frac{1}{2}H_0^{-1}$	$t_0/2$	4.4×10^9
Dirac additive creation	H_0^{-1}	t_0	1.8×10^{10}
Dirac multiplicative creation	H_0^{-1}	----	----

lack of long period pulsars independently of any details of pulsar magnetospheres.

An exact solution of equation (5) requires a numerical integration and is dependent on the details of the cosmological model through $H(t)$ which increases slowly with time⁸. Considerable insight can, however, be obtained by letting $4n\dot{\gamma}/\gamma^{-1}(3-n)^{-1} \simeq 15H_0$ in the last term in equation (5). This overestimates the term at early times when it is unimportant and underestimates it slightly for $t > t_0$. When the magnetic flux through the poles is conserved the first term in equation (5) is constant. Then

$$(P/P_c)^2 = 1 - \exp(-15H_0\tau) \quad (7)$$

where τ is the time since the pulsar was born. For $15H_0\tau \ll 1$, equation (7) reduces to the standard expression for $P(\tau)$. The effect of an increasing γ is only important for $\tau \gtrsim 5 \times 10^6$ yr. Thus, only a small percentage of the oldest pulsars in the Galaxy would approach the limiting condition $\dot{P} = 0$ at $P = P_c$. For JP1953 the age implied by equation (7) would be $\tau = 1.8 \times 10^9$ yr rather than $\tau = 4.3 \times 10^9$ yr on the standard theory.

The above criticism of the G -changing cosmologies, derived on the basis of zero electromagnetic losses, is independent of the vagaries of magnetospheric models and loss rates. The model used to find $(P/\dot{P})_{\max}$ when $\gamma \propto t^{-1}$ is quite adequate for the old low mass pulsars considered. Therefore the values in Table 1 should be a firm upper limit for $P/\dot{P}$ with the major uncertainty coming from the uncertainty in H_0 . Of course, this analysis also applies to spinning white dwarfs.

JP1953 could be a much more exotic object than assumed or could participate in some very implausible orbital effects. These possibilities have a very small but finite probability and will be examined in detail in a forthcoming paper.

The critical period, $P_c \simeq 5$ s, derived from the group theory result with $\dot{\gamma}/\gamma = 3H_0$, is not strongly dependent on relativistic gravity either since $P_c \propto (I/I)^{1/2}$. The uncertainties here come from the fairly large range in permissible pulsar parameters and there seems to be no clear way to narrow the range.

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ANS observations on the X-ray burster MXB1730–335

A NEW type of time variability of cosmic X-ray sources ('bursters') was discovered from Astronomical Netherlands Satellite (ANS) observations on the source 3U1820–30, associated with the globular cluster NGC6624 (refs 1, 2). These X-ray bursts are characterised by a rapid rise in flux (~ 0.5 s) to a relative intensity comparable to the Crab Nebula, followed by an exponential decay at a time scale of ~ 10 s. Certain characteristic features of the bursts, in particular the shape and spectral hardening, are consistent with a model where the X-ray emitting region is situated in a cloud of hot gas near a massive black hole ($\sim 1,000M_\odot$) (ref. 3). On March 14, 15 and 16, 1976 the ANS spacecraft was pointed in the direction of the remarkable new X-ray burster MXB1730–335 discovered by SAS-3 (ref. 4) in the constellation Scorpius at 5° from the galactic centre in the galactic plane. The source is characterised by rapidly repetitive X-ray bursts of varying intensity. The energy in a given burst is approximately linearly proportional to the time interval to the next burst⁵. We report here an improved position of this source and give the energy spectrum from data obtained in four observations for which quick-look data are available. An upper limit for the steady source level in between the bursts is obtained.

In Fig. 1 the counting rate histogram is shown for two observations, from the Utrecht experiment SXX, in the energy range 1–7 keV. Spectral information is available per second in the 1–7 keV range (SXX) and every 4 s in the 1.3–30-keV range for the Cambridge experiment HXX. The steady source intensity in these data is < 1 count s^{-1} or ~ 15 Uhuru counts s^{-1} ($\lesssim 3.10^{36} \times d_{10}^2$ erg s^{-1} in the range 1–7 keV, where d_{10} is the distance (to the source) in units of 10 kpc). We observed the source when it was emitting bursts at an almost constant peak intensity of 16 counts s^{-1} on March 14.639 (Fig. 1b) and 12 counts s^{-1} on March 14.173 (Fig. 1a) or ~ 0.2 and 0.15 times

Fig. 1 Two examples of counting rate histogram (Utrecht SXX experiment) of the burster source MXB1730–335 in the energy range 1–7 keV. a, March 14.173; b, March 14.639. Integration time (1 bin) is 1 s.

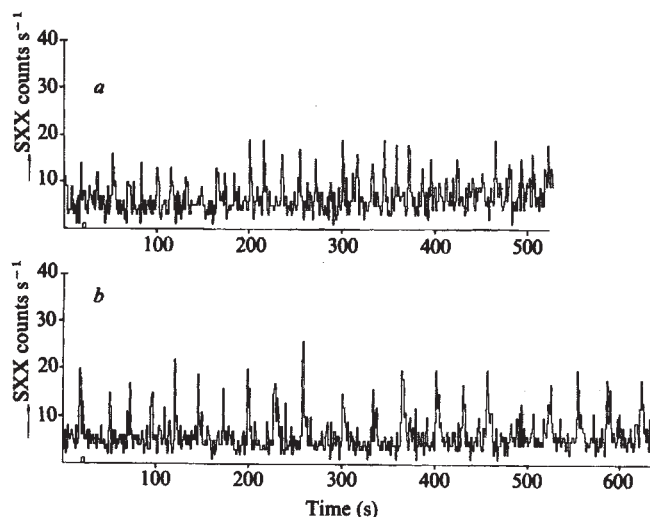


Table 1 Data on X-ray bursters

Source	Discovered by	Position (1950.0)	Area of error box (degree)	Remarks	Reference
NGC6624	ANS	18 h 20.5 min −30°23′	0.033	Bursts at intervals of ~0.18 d	1, 2, 5, 14
In Norma	Vela-5	16 h 07 min −53°	~10		12
9 events A through I	Vela-5		~100		12
MXB1743–293	SAS-3	17 h 42.6 min −29°16′	0.01	Bursts at intervals ~0.55 d	16–18
MXB1742–297	SAS-3	17 h 41.7 min −29°40′	0.01	Bursts at intervals ~1.46 d	16–18
NGC1851	Uhuru	05 h 12.4 min −40°05′	5		15
In Aquila	SAS-3	19 h 29 min +8°	~150		23
MXB1730-335	SAS-3	17 h 30 min 08 s −33°21.5′	0.0022	Frequent bursting, associated with globular cluster	4–9 this paper
MXB1728-34	SAS-3	17 h 28 min 29 s −33°48′	0.0035	Bursts at intervals ~0.20 d	4, 5
In Puppis	SAS-3	07.6 h −50°	~170		24

the intensity of the Crab Nebula. The rise time of the bursts is $\lesssim 1$ s, a typical characteristic of the bursters. The duration of the bursts varies and is typically a few seconds. The average time between the bursts is 26 s and 16 s, respectively, and the total energy per burst (1–7 keV) is $\sim 10^{38} d_{10}^2$ erg and $\sim 8 \times 10^{37} d_{10}^2$, respectively. This is consistent with the relationship found by Lewin *et al.*⁵ for the energy per burst and the time separation between the bursts over a somewhat different energy range. We integrated 20 bursts to obtain a signal-to-noise ratio sufficient for a determination of the position and the spectral parameters.

The position of the source is obtained by comparing counting rates of the various X-ray instruments aboard ANS. The two $3^\circ \times 10'$ FWHM field of view collimators of the Cambridge experiment HXX, which are mutually offset with $3.7'$ have a count-rate ratio for 20 integrated bursts of 2.20 ± 0.16 . This determines the declination of the source (90% confidence level) to be $2.5 \pm 1'$ north of the pointing direction used, which was the most probable SAS-3 position⁶, supplied by W. Lewin before publication. Hence $\delta(1950.0) = -33^\circ 22.5' \pm 1'$. The ratio of the counts seen in the HXX and SXX detectors confines the source to the central position of the field in right ascension ($\pm 30'$). The position thus obtained⁷ is plotted in Fig. 2, together with the SAS-3 position. The region of intersection ($2' \times 3'$) of the possible positions for the source MXB1735–335 reveals a faint, highly reddened star cluster, probably globular, with coordinates $\alpha = 17$ h 30 min 08 ± 01 s, $\delta(1950.0) = -33^\circ 21' 25 \pm 10''$ (ref. 8). Also the position found with the rotation modulation counters aboard Ariel V (ref. 9) is consistent with the cluster being the source MXB1730–335.

The integrated X-ray burst spectrum, obtained by adding 20 bursts after subtracting the background before and after each burst, is characterised for an exponential fit by kT of 10^{+3}_{-2} keV and a photoelectric absorption, from a hydrogen column density of $5^{+2}_{-1} 10^{22}$ atoms cm^{-2} (using Brown and Gould¹⁰ abundances). (Quoted errors are 1 s.d.) This value for the hydrogen column density N_H taken together with the estimated visual absorption⁸ for the globular cluster $A_V = 15$ mag, matches quite well with the gross average for the Galaxy for this ratio. $N_H/A_V = 2.2 \times 10^{21} \text{ cm}^{-2} \text{ mag}^{-1}$ as derived in ref. 11. This implies that the low energy absorption cutoff of the burster is consistent with the interstellar absorption found for the globular cluster. Separate spectra for the peak and the decay phase of the bursts show evidence for a spectral hardening during the burst. This feature will be studied further, when all (production) data for this source will be available.

We have therefore observed the same rapidly repetitive X-ray burster that MIT reports⁵, based on positional agreement and

general X-ray characteristics. The improved positional error box of this source ($2' \times 3'$) contains a globular cluster and provides thus another example of an X-ray burster that may be related to globular clusters. The consistency between the interstellar absorption measured from the burster and the optical extinction measured from the cluster further provides evidence for the identification.

At about the same time of the ANS discovery of an X-ray burster in NGC6624, a report from 15 months of operation of the two Vela-5 satellites showed 20 X-ray bursts of duration > 2 s and < 128 s (ref. 12). Ten of these are consistent with one position in the constellation Norma and lie close to a transient X-ray source found by Uhuru (ref. 13). Subsequently many more bursts have been found in data from the SAS-3, Uhuru and Ariel V satellites. Clark¹⁴ found 11 bursts in SAS-3 data from a region of the sky of $12^\circ \times 12^\circ$ containing the globular cluster NGC6624, exhibiting the same characteristic time structure and spectral hardening as the ANS bursts and thus most probably originating from the same globular cluster. The bursts show a mean time separation of 4.3 h with a 4% r.m.s. deviation. It is probable that during SAS-3 observations in January 1976 on 3U1820–30, when the source was twice the maximum Uhuru intensity no X-ray bursts were observed⁵, suggesting indeed that the feature of X-ray bursts in NGC6624 is related to the intensity level of 3U1820–30.

In the old Uhuru data an X-ray flare of duration > 15 s and < 6 min was found¹⁵ in a region of $0.5^\circ \times 10^\circ$ containing the globular cluster NGC1851. Although the complete time structure, in particular the rapid rise time, could not be detected from a spinning satellite, the observed spectral hardening during the event indicates that one is probably dealing indeed with the same type of X-ray bursts.

Two sources of X-ray bursts, designated MXB1743–293 and MXB1742–297, were found by SAS-3 within a degree of the galactic centre^{16–18}. The bursts show a mean time separation of 1.46 and 0.55 d, respectively. The bursts from MXB1742–297 seem to show in most cases a double pulse structure at the higher energies (8–18 keV). A summary of the newly discovered bursting X-ray sources is given in Table 1.

The nature of the bursting X-ray sources thus seems to be quite different from sources identified so far as binary X-ray stars. The importance of this was already stressed by Grindlay *et al.*². At least three of the dozen now known bursters are identified with globular clusters (NGC6624, NGC1851 and Liller's globular cluster). Three others (MXB1743–293, MXB1747–297 and MXB1728–34) originate in the central region of the Galaxy and could very well be related to completely obscured globular clusters. The remaining bursters have

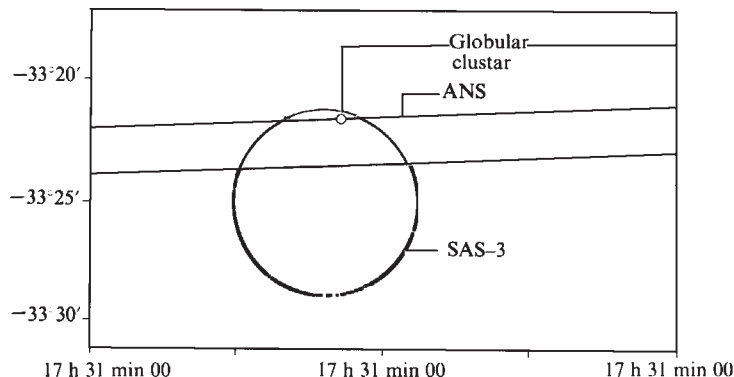


Fig. 2 Positions obtained by various satellites of the X-ray burster MXB1730-335. In the intersection of the error boxes a newly discovered globular cluster is present.

positions that are too poorly defined to make any definite conclusion regarding identification. Two bursters (NGC6624, NGC1851) are also known X-ray sources (3U1820-30 and MX0513-40) that belong to a group of seven sources associated with globular clusters.

The correlation between the intensity level of 3U1820-30 and the existence of X-ray bursts in that source, indeed suggest a physical relationship between the two. Several authors have pointed to the intrinsically different nature of globular X-ray sources. This follows, for example, from the ratio of the X-ray luminosity, which is one of two orders of magnitude larger than the corresponding ratio for the entire Galaxy or the galactic bulge²⁰. The majority of these seven X-ray sources are associated with globular clusters that have high central escape velocity and are centrally condensed. This has led various authors^{21,22} to suggest that the X-ray producing mechanisms somehow are related to the existence of massive remnants of evolved stars and possibly with massive black holes of ~ 100 – $1,000 M_{\odot}$. This is consistent with such a massive black hole if the decay phase of the burst is interpreted as Compton scattering from a hot cloud around the hole.

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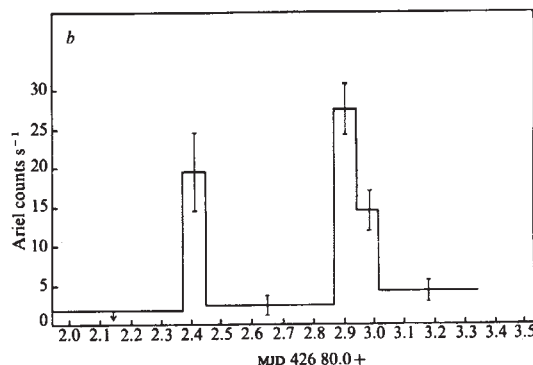
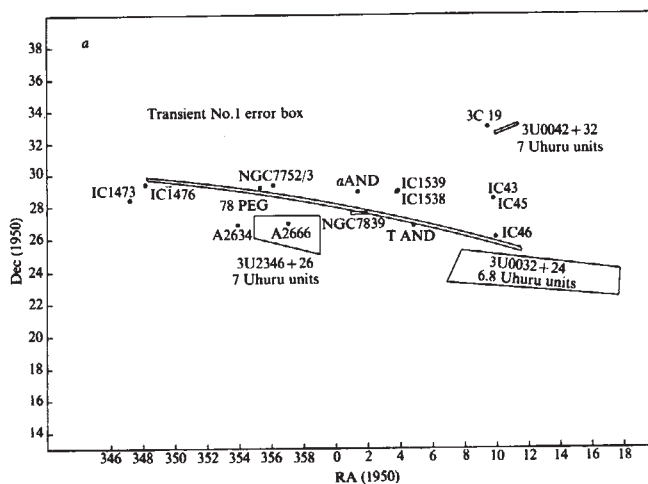
Two short lived X-ray transients at high galactic latitude

UNTIL recently, sources known to exhibit transient behaviour were restricted to a region close to the galactic plane. Now, extended observations at high galactic latitudes by the Ariel V, ANS and SAS-3 X-ray detectors have revealed transients with an impressive variety of temporal behaviour. Reporting the first such transient Ricketts *et al.*¹ described how A1103+38 ($b^{11} \approx 65^\circ$) increased in intensity by an order of magnitude over a period of between 10 and 28 d with an additional 'flare' which lasted ~ 1 d. In contrast Rappaport *et al.*² observed a spectrally hard transient MX2346-65 ($b^{11} \approx -51^\circ$) which lasted between 45 s and 2,200 s (ref. 2) and was about as intense as the Crab Nebula. In the error box of A1103-38 the radio source B2 1101+38, whose optical counterpart MK421 may be a BL Lacertae object, could be responsible for the X-ray transient. As there are no obvious optical candidates with which to associate MX2346-65, Rappaport *et al.* suggest sporadic mass transfer on to a white dwarf from a faint, variable dM class star. We here report two transients at high galactic latitudes observed by the Leicester sky survey experiment on Ariel V whose time scales, of the order of hundreds of minutes, are intermediate between those discussed above.

Besides their intrinsic interest, it seems possible that these various high latitude transients have some bearing on the less well determined, mainly unidentified high latitude X-ray sources in the third Uhuru catalogue, some of which may also be associated with relatively nearby galactic objects.

The first new Ariel V transient source, observed on September 27, 1975, was in a region of sky close to Uhuru

Fig. 1 a, Error box (90% confidence in width, full collimator dimension in length) for the transient A0000+28 of September 27, 1975. b, Light curve of transient A0000+28 for source at centre of error box. Error bars are $\pm 1\sigma$ from counting statistics.



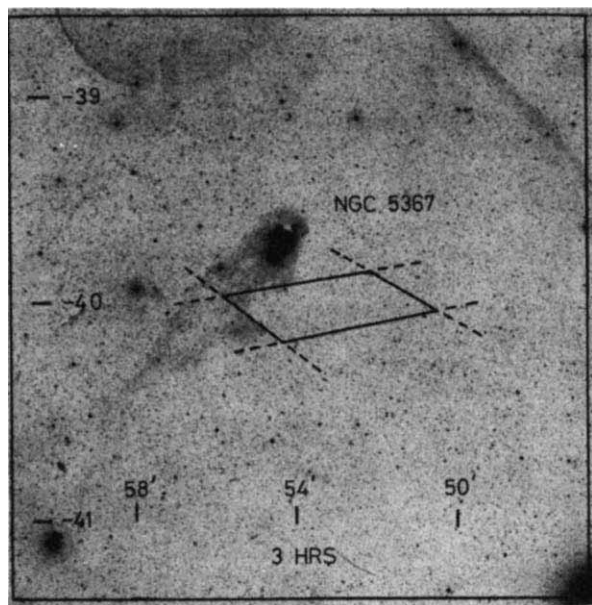
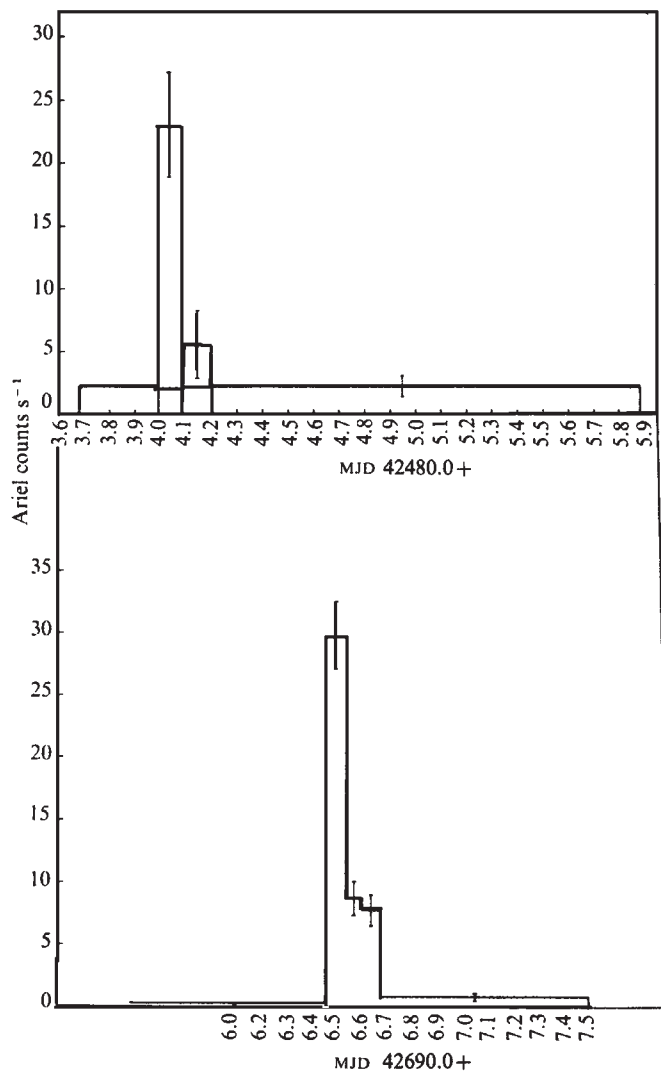


Fig. 2 *a*, Superposition of error boxes of A0353-40 transients of March 12 and October 1, 1975 on Sky Survey plate. Coordinates are 1950. Photograph reproduced by courtesy of SRC Schmidt Telescope Unit. *b*, Transient A0353-40 light curves assuming common origin of X-ray emission near NGC5367. Error bars are $\pm 1\sigma$ from counting statistics.



sources 3U2346+26, 3U0042+42 and 3U0032-24. The long thin error box (Fig. 1*a*) which results from a single observation of the transient, contains no obvious optical or radio candidate for association except for T And, an M3 type, Mira variable, whose visual magnitude varies between 7.7 and 14.3 with a period of 280.91 d (ref. 3). Fabian, Pringle and Webbink⁴ discussed the possibility of transient X-ray emission from Mira variables in relation to the Cen Xmas source A1118-61. The light curve of the September 27 transient is shown in Fig. 1*b*, corrected for an assumed source position at the centre of the error box. The light curve shows an initial peak (related to precursor peak common to the galactic plane transients⁵?), followed by a main peak lasting about three satellite orbits (~ 5 h).

An unsuccessful search through Ariel V data for other appearances of the transient gave a good opportunity to investigate the nearby 3-U sources. If still at their catalogued strength (~ 6 Uhuru units in each case) each should have been observed with high significance ($\sim 5\sigma$) at least twice in the Ariel V data. The non-observation of any of the sources places the strengths at no more than 2 Uhuru units and suggests a variability that makes unlikely the association of 3U2346+26 with the Abell clusters A2666 or A2634 (ref. 6).

The second new transient was noticed in Ariel V quick look data on October 11, 1975, and found to be in the region of 3U1439-39 and Cen-A. Examination of earlier sky survey data for this region revealed a transient ~ 200 d earlier. From the number of high latitude transients which have been observed by the Ariel V sky survey experiment (five since launch), the probability that what has been observed comes from 2 different

transients is only 10^{-3} . The error boxes intersect (Fig. 2*a*) and both light curves in Fig. 2*b* have been calculated by normalising the counts as from an object at the centre of the combined error box. Their similarity with respect to amplitude and time scale further suggest that the same transient has been observed twice. Neither the error box, nor the field close by contain any reported variable stars³ or unusual extragalactic objects; all the stars in the error box are fainter than $M_v = 9$. There is an $M_v = 6.3$ double star 18936 in Aitken's catalogue at 3 h 58 min, -39.9° .

Near the edge of the transient error box is the impressive, bright reflection nebula NGC5367. From a bright diffuse head containing embedded stars a diffuse tail extends nearly one degree towards the galactic plane. The cometary appearance of NGC5367 and similar objects in the region of the Gum, and possibly Orion, nebulae have earned them the description 'cometary globules'. However it has been initiated in such isolated clouds, star formation clearly is taking place in NGC5367, the obvious embedded stars being of mid to late B type⁸. Such a structure should be expected to contain also possibly T Tauri stars⁹ and analogously with the Orion and Taurus T associations also UV Ceti flare stars. Predictions of X-ray flare intensities from UV Ceti stars have already been made by scaling up from the stellar radio emission during a flare using the ratio between X-ray and radio fluxes observed in solar flares¹⁰. To date, no X-ray flares from UV Ceti stars have been detected¹¹ though long term correlated radio, optical and X-ray observations have been made (C. J. Crannell *et al.*, unpublished) and are being analysed. For an $M_v = 1$ flare on UV Ceti the most optimistic X-ray flux in the range of the

Ariel V sky survey experiment is $\sim 10^{26}$ J (ref. 10) which is to be compared with a peak emission of $\sim 10^{27}$ J from NGC5367 assuming its distance to be 300 pc (ref. 8). If NGC5367 is the origin of the transient X-ray emission it would repay study for significant variability in other parts of the spectrum.

I am grateful for discussions with Dr P. W. J. L. Brand concerning NGC5367 and for his provision of an overlay for Fig. 2a. The Ariel V project is supported by the SRC.

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Statistical interpretation of enthalpy-entropy compensation

TYPICALLY, enthalpy and entropy estimates from kinetic and equilibrium data are highly correlated, varying in a linear fashion with one another (enthalpy-entropy compensation effect). This effect can be readily explained in most cases simply as a statistical or data handling artefact. The statistical analysis presented here reveals three novel insights. First, the enthalpy and entropy parameter estimates are highly correlated, such that estimated correlation coefficients > 0.95 , say, do not imply chemical causation. Second, enthalpy and entropy estimates are distributed by experimental and measurement errors in elliptical probability regions that are very elongated and appear as lines. The slope of such lines is the harmonic mean of the experimental temperatures. Third, estimates of enthalpy and free energy at the harmonic mean of the experimental temperatures are not statistically correlated, so any observed structured variation between these parameter estimates arises from the chemical effect alone. Note that, since the thermodynamic potentials are interrelated by the Maxwell relationships, a correlation between any two potentials can be transformed to give the corresponding correlation between any other two. We now discuss these results to resolve a number of issues concerning a much disputed data set.

Many linear enthalpy-entropy data sets have been catalogued for which the correlation coefficient > 0.95 (refs 1, 2). Implicit in such efforts is the hope that the high correlations imply the detection of a true chemical effect. For data on the oximation of alkyl thymyl ketones³, for example, the correlation coefficient is 0.9724 and without the methylated compound it is 0.9988. It is not surprising, then, that these data have been rather widely discussed as perhaps displaying a linear compensation relationship^{1,2,4-6}. Since the data were taken over a relatively narrow temperature range (30, 35 and 40 °C) the estimates are highly correlated. In fact the correlation between parameter estimates, even in the absence of any chemical compensation effect, is

$$\rho = \frac{\text{Cov}(\Delta\hat{H}^\ddagger, \Delta\hat{S}^\ddagger)}{[\text{V}(\Delta\hat{H}^\ddagger)\text{V}(\Delta\hat{S}^\ddagger)]^{1/2}} = \frac{\Sigma 1/T}{[\Sigma (1/T)^2]^{1/2}} = 0.99991$$

Thus one cannot infer that the high observed correlation between enthalpy and entropy parameter estimates is not just a

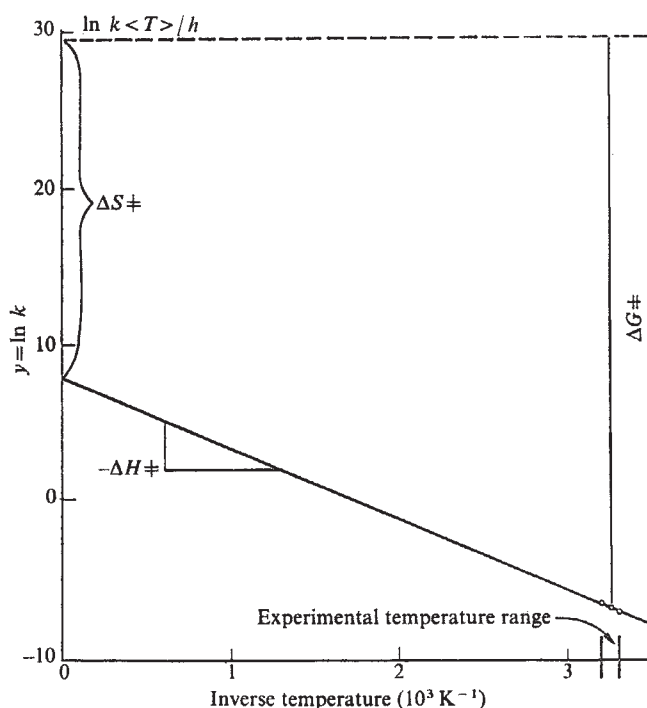


Fig. 1 Geometric interpretation of the parameter estimates. The indicated lengths are proportional to $\Delta G^\ddagger$ and $\Delta S^\ddagger$ and the indicated slope is proportional to $-\Delta H^\ddagger$. These data for the oximation of methyl thymyl ketone³ indicate a strong dependence of the intercept estimate on the slope estimate because the data were taken over a very small temperature range far from the origin. The three data points are designed by dots.

result of the deviations caused by errors. That the enthalpy and entropy estimates should be so highly correlated is displayed in Fig. 1. The measure of enthalpy is the slope of a regression line on an Arrhenius or van't Hoff plot. And the measure of entropy is the intercept at $1/T = 0$. Thus experimental or measurement errors will cause a slightly imprecise regression line with an error in the intercept measure 'compensating' the error in the slope measure. The intercept measure which is independent of slope deviations, however, is the one at the arithmetic mean of the independent variable, $1/T$, the inverse experimental temperature. Thus a useful reformulation of the Arrhenius equation is one that is centred about the average inverse experimental temperatures $\langle 1/T \rangle$

$$\ln k = \ln A - E/RT = (\ln A - E/RT_{\text{hm}}) - E/R(1/T - \langle 1/T \rangle)$$

Since this new intercept estimate is uncorrelated with the slope estimate, the correlation between enthalpy and free energy estimates at $T = T_{\text{hm}}$ is zero

$$\rho_{\Delta\hat{H}, \Delta\hat{G}_{T_{\text{hm}}}} = 0$$

In particular the correlation between enthalpy and free energy estimates for the alkyl thymyl ketones is -0.2273 for the entire data set and 0.0770 for the data without the methylated compound. Since these correlations are not at all significant with just 7 and 6 data pairs, respectively, we conclude that no significant correlation between thermodynamic parameters is detected and the observed correlation in the enthalpy-entropy plane arises from the statistical propagation of errors as a data-handling artefact.

Much confusion has existed over how to estimate a line from data plotted in the enthalpy-entropy plane. To illustrate this, the thermodynamic parameter estimates for the oximation of alkyl thymyl ketones were plotted in Fig. 2 along with the

corresponding 50% confidence regions. Since both the form of the Arrhenius equation and the transformed Arrhenius equation given above are linear, the confidence regions are elliptical. In particular, the ratio of major to minor axes in the enthalpy-entropy plane is 23,252:1. Thus the distribution of data is well characterised by merely the major axis of such a region, which is determined from a canonical analysis to be

$$\Delta H = T_{hm}\Delta S + \Delta\hat{G}_{T_{hm}}$$

and which differs from the postulated chemically caused line

$$\Delta H = \beta\Delta S + \Delta G_\beta$$

by the value of the slope parameter β , the compensation or isokinetic temperature^{1,2,7,8}. The compensation temperature is commonly determined by least squares. The authenticity of a chemical compensation has been questioned when the value of the compensation temperature approaches that of the experimental temperatures^{1,5,6,9-12}. We maintain that since the two relationships differ only in the slope parameter it is sufficient to test the value of that parameter against the harmonic mean of the experimental temperatures to determine if a chemical effect

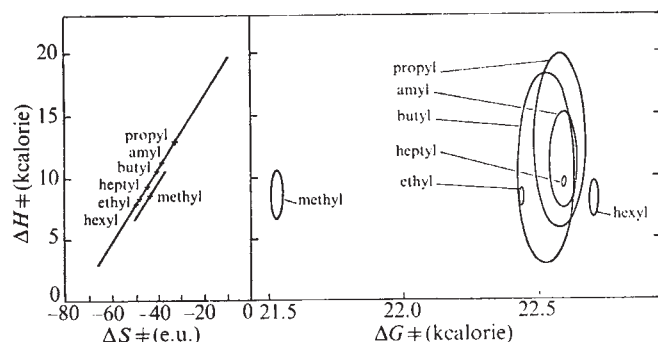


Fig. 2 The 50% joint confidence regions for the oximation of alkyl thymyl ketones⁶. The $\Delta H^\ddagger - \Delta S^\ddagger$ ellipses are so narrow that they appear as lines. Departure of the methylated compound from a common $\Delta G^\ddagger$ value causes it to fall off the statistical compensation 'line' between $\Delta H^\ddagger$ and $\Delta S^\ddagger$ estimates. Notice that $\Delta G^\ddagger$ is estimated more precisely than $\Delta H^\ddagger$. All values were calculated for $T = T_{hm} = 308.1$ K.

is detectable beyond the statistical compensation line. Furthermore, in the limit that the ratio of axes in $\Delta H - \Delta S$ coordinates is very large, lack of fit to the statistical compensation line is just the error in the estimate of $\Delta G_{T_{hm}}$, which is distributed with zero mean and constant variance, justifying application of least squares to estimate a slope for comparison with T_{hm} . For the oximation of thymyl ketones, a 95% confidence interval for β is (226, 401) and without the methylated compound is (286, 328) where T_{hm} is 308 K. Thus no chemical effect was detected, as shown previously by the correlation analysis.

We have also tested 37 other data sets from several reviews^{1,2,5,6,8,9,13} and for only three of these was the hypothesis rejected that the observed compensation pattern can be explained as an artefact. Only data sets that were thermally consistent, allowing computation of a unique T_{hm} , were tested.

From this analysis we conclude that high correlations of enthalpy-entropy estimates usually do not reflect any chemical causality but correlations between enthalpy-free energy estimates evaluated at T_{hm} do imply chemical causality. Least squares regression of enthalpy estimates on entropy estimates is only useful to determine if a chemical factor is causing noticeable deviation from the statistical correlation pattern

but does not give unbiased estimates of compensation temperatures.

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Age of the lower flood basalts of the Ethiopian plateau

VARIOUS stages of continental fracturing and crustal separation are displayed in Ethiopia at present. Separation of the African and Arabian plates has taken place along the Red Sea and Gulf of Aden rifts and in several areas of Afar where axial volcanic ranges are composed of low-potassium oceanic tholeiites¹. The onset of spreading was accompanied by deep faulting and intense fissural activity, with the eruption of very fluid flood basalts over Mesozoic sediments and Precambrian basement. The flood basalts outcrop on the plateaux flanking the rift system (Fig. 1).

The determination of radiometric ages from the flood-basalt succession is important in the reconstruction of the tectonic and magmatic events which preceded rifting. Following the dating of basal flows from four localities², 22 ages have been determined from flows higher in the sequence and from other localities, shown in Fig. 1. These results show the time range during which the lavas were erupted and they also provide a stratigraphic control on the validity of the basal ages. K-Ar ages were measured on whole-rock samples and the new data are presented in Table 1, together with the brief petrographic descriptions. Errors quoted are at the 60% confidence limit and based on replicate analyses.

With the exception of one sample (30940) an excellent stratigraphic consistency is maintained throughout the profiles and the age ranges are shown in Fig. 2, which is a compilation of the new data reported here and those reported before²⁻⁴. The age of sample 30940 has been omitted from Fig. 2 because the other ages from that profile suggest that sample 30940 has suffered argon loss. In only one profile examined do the basalt ages exceed 30 Myr, that is the Wolkefit Pass profile in the Simien massif (36 Myr). This supports the conclusion² that the major part of the plateau lavas is much younger than previously thought. In a paper by Mohr⁵ (Fig. 1) a histogram of all other available age data also supports this conclusion, and it is interesting to note that Mohr has omitted the older ages of Megrue *et al.*⁶ due to geological inconsistencies, as well as omitting his previous data from the Blue Nile Gorge⁷ which have been shown to be erroneous^{2,3}. Although it cannot be stated definitely that any of the flows dated in this work represented the oldest volcanics of the province, some of the data disagree with the sequence produced by Zanettin *et al.* which extends to much older ages⁸⁻¹⁰. Specific examples of these inconsistencies are in the Blue

Table 1 Sample data

Sample no.	Profile	Position in profile	%K	Volume ^{40}Ar radiation standard $\text{cm}^{-3} \text{g}^{-1} \times 10^{-9}$	% ^{40}Ar radiation	Age (Myr)	Petrography
30406	Aisha	Upper flow	0.67	0.5375	69.3	19.8 ± 0.7	Coarse olivine and pyroxene-phyric basalt with matrix of plagioclase, pale brown augite, and magnetite. Fine-grained pilotaxitic basalt. Sparse plagioclase phenocrysts in a quenched matrix of plagioclase, clinopyroxene and opaque ore.
30405		Lower flow (tilted traps)	1.11	0.8877	66.8	19.9 ± 0.7	
30412	Jijiga	Sheet in sandstones	0.38	0.3062	28.1	20.1 ± 0.7	Medium-grained dolerite with clinopyroxene and plagioclase phenocrysts in matrix of similar composition plus olivine and opaques. Clusters of zoned plagioclase phenocrysts in fine-grained matrix, with approximately 8% brown isotropic glass.
30414		Top flow	0.76	0.6911	39.8	22.6 ± 0.7	Fine aphyric basalt. Plagioclase (An_{62}), augite and quenched matrix with dusty opaque ores.
30437	Cassam	Top basic flow	1.14	0.4067	61.0	8.9 ± 0.3	Euhedral plagioclase phenocrysts ($\text{An}_{68}\text{--An}_{55}$) in similar matrix as above with 5–10% isotropic glass. Similar to 30437. Intergranular matrix.
30444		130 m from top	1.11	0.3904	59.4	8.7 ± 0.3	Approximately 12% pyroxene and olivine phenocrysts in an intergranular matrix of plagioclase, clinopyroxene, and opaque ore.
30446		150 m from top	0.88	0.3464	53.2	9.8 ± 0.3	Pyroxene-phyric basalt with quenched matrix containing opaque ore laths. Approximately 10% glass.
30878	Maichew	140 m from base	0.92	0.9691	79.1	26.2 ± 0.8	Olivine dolerite. Slightly serpentinised olivines in ophitic matrix of plagioclase, opaque ore and pinkish augite.
30875		110 m from base	0.97	1.0003	85.6	25.7 ± 0.8	Dense black microporphyrritic basalt with feldspar laths in a pilotaxitic matrix containing abundant opaque ore.
30947	Adigrat	Dyke	0.28	0.1746	39.2	15.5 ± 0.5	Aphyric basalt. Pinkish-brown augite, rods of opaque ore and ragged feldspar laths.
30905		Top flow (640 m)	0.92	0.6962	38.1	18.8 ± 0.6	Aphyric patchy texture of aligned feldspar laths in subophitic augite and greenish quench. Sparse opaques.
30908		480 m from base	0.92	0.7397	47.3	20.1 ± 0.7	Fine-grained aphyric basalt. Plagioclase ($\text{An}_{58}\text{--An}_{55}$), pale brown augite and opaque ore. < 5% isotropic glass.
30895		100 m from base	0.21	0.1879	39.2	22.4 ± 0.7	Plagioclase and clinopyroxene phenocrysts in a cryptocrystalline quenched matrix.
30911	Garamullatta	610 m from base	1.27	1.0573	73.3	20.8 ± 0.7	Subophitic basalt. Plagioclase laths, small olivines and euhedral opaque ore in clinopyroxene.
30927		360 m from base	0.71	0.5895	61.7	20.7 ± 0.7	Sparsely porphyritic basalt. Clinopyroxene phenocrysts, zoned at margins, in plagioclase-clinopyroxene-opaque ore matrix.
30942		180 m from base	0.78	0.7597	62.8	24.3 ± 0.7	Clinopyroxene, olivine, and plagioclase phenocrysts in a pilotaxitic matrix of similar composition.
30971	Mugher	300 m from base	1.13	0.8967	83.6	19.8 ± 0.7	Fairly coarse basalt. Plagioclase (An_{65}), altered olivine, clinopyroxene and opaque ore in seriate texture.
30967		260 m from base	1.00	0.8612	81.0	21.5 ± 0.7	Approximately 40% large plagioclase laths in a matrix of pinkish-brown clinopyroxene, plagioclase and opaque ore. 10% isotropic glass.
30958		180 m from base	1.14	0.9932	81.4	21.6 ± 0.7	Clusters of plagioclase phenocrysts in similar matrix as below.
31000	Wolkefit Pass	1,150 m from base	1.04	0.9526	77.1	22.8 ± 0.7	Big-feldspar basalt. Zoned plagioclase phenocrysts in quenched matrix of plagioclase, brown augite and approximately 10% opaque ore.
30999		1,030 m from base	0.46	0.4985	48.9	27.0 ± 0.8	Feldspar-phyric basalt. Approximately 15% plagioclase ($\text{An}_{65}\text{--An}_{55}$) in holocrystalline pilotaxitic matrix.
30991		760 m from base	1.19	1.5130	23.6	31.6 ± 0.9	
30988		300 m from base	0.47	0.6806	49.6	36.0 ± 1.1	

$\lambda_e = 0.584 \times 10^{-10} \text{ yr}^{-1}$; $\lambda\beta = 4.72 \times 10^{-10} \text{ yr}^{-1}$; $^{40}\text{K} = 1.19 \times 10^{-11} \text{ mol per mol K}$.

Nile and Mugher Gorge profiles. The Blue Nile volcanics, classified by Zanettin as Aiba Basalts (34–30 Myr), have been dated in Canberra giving ages between 27 and 23 Myr (ref. 3). Mugher Gorge basalts, placed in the Ashangi (older than 34 Myr) and Aiba (34–30 Myr) groups by Zanettin *et al.*, have been dated in Leeds between 22 and 19 Myr (work reported here). Due to these obvious discrepancies the chronological sequence of Zanettin *et al.* cannot be confirmed.

There is excellent agreement between the data from the south-eastern profiles (Jijiga and Garamullatta) and work by Morbidelli *et al.*¹¹ and Kunz *et al.*¹² from localities further west on the same plateau. All the data indicate a maximum age of approximately 25 Myr for the onset of widespread volcanism on the south-eastern plateau.

Figure 2 shows that there is a general decrease in the

ages of the profiles from north to south, indicating a possible southward migration of the initial activity. This idea of southward younging was first mentioned by Dainelli¹³ and later by Mohr¹⁴ and Gass¹⁵, although they believed it began much earlier in the Eocene epoch. Zanettin *et al.*¹⁰ have also demonstrated the migration of the volcanism towards the rift zone. This apparent progression towards the rift is caused by the gradual subsidence of the area during volcanic activity, resulting in the restriction of the flows to progressively narrower zones. A similar process has affected the Iceland flood basalts (I. L. Gibson, personal communication).

The Jijiga profile provides an interesting date from the intrasedimentary basalts (30412). From field relationships it is uncertain whether the basalts are flows within the sandstones, and therefore of Mesozoic age, or younger

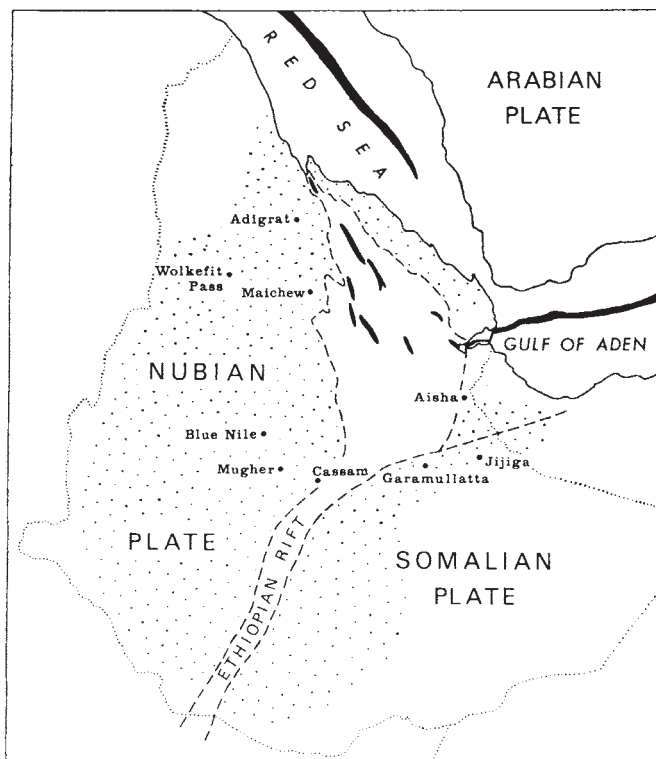
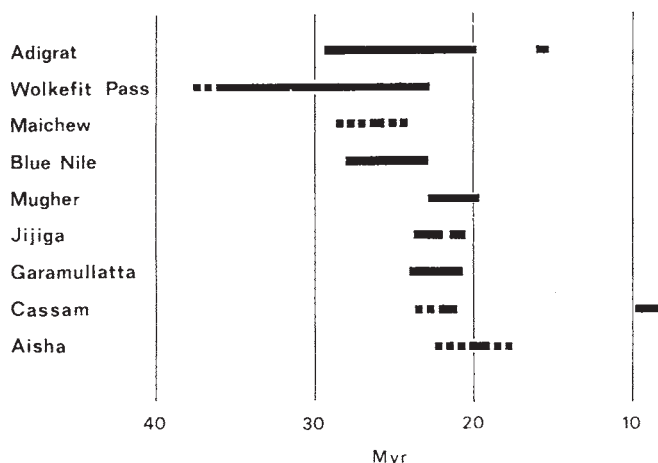


Fig. 1 The rift system in Ethiopia. Black areas, zones of generation of new oceanic crust. Stippled areas, Ethiopian plateaus. Solid circles, profile localities.

intrusives. Mohr¹⁴ considered these to be Mesozoic flows. The radiometric age of 20.1 Myr shows that they are intrusive sills emplaced during the profuse igneous activity at that time.

An important conclusion from all these new data is that there was widespread eruption of flood basalts over the plateau areas during the period 25–19 Myr ago (Fig. 2). It had been proposed¹⁵ that the trap basalts represent a separate stage of volcanism which preceded the formation of the Afar depression. But according to Barberi *et al.*¹⁶ the Afar depression began to form approximately 25 Myr ago, and there is therefore a large overlap of ages from the plateau and Afar regions. This shows that flood basalts were being erupted on the plateaus even during the formation of the depression, and that igneous activity was contemporaneous in the two regions for a long time. The

Fig. 2 Age ranges obtained from the nine profiles. Broken lines indicate where the plateau basalts continue above or below the measured profile.



volcano-tectonic sequence during the initial fragmentation of the continental plate in north-eastern Africa must be accommodated within a time range which is much shorter than in previous models^{14,15}.

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The Marda Fault Zone, Ethiopia

THE Marda Fault Zone in south-eastern Ethiopia was first recognised in the Marda Range near Jijiga (Fig. 1) and was called the Marda Hills line. The “linear NW–SE arrangement of basalt capped summits with basaltic plugs” and the associated Bouguer anomaly were considered indications of a major “volcanic-tectonic” lineament¹. Subsequently the fault zone was described as a complex of NW–SE trending faults, down thrown to the NE and possibly extending 200 km into the Ogaden Basin². Recent studies have indicated that the fault zone extends over 400 km beyond the Marda Range to the Belet Uen area in Somalia³. Moreover, a major fault zone trends south-east from Belet Uen to the Somalia coast and can be considered a further extension of the zone⁴. These indications of a zone of faulting from the southern margin of the Afar Depression south-east to the Indian Ocean define a length for the Marda Fault Zone of >900 km. The feature must therefore be recognised as a major structural element in the Horn of Africa. We here attempt to define the structure and age of the zone, and have made use of ERTS-1 (LANDSAT) Band 7 imagery, supplemented locally with colour composites, detailed photostudy between 7°50'N and 9°00'N, and potential methods surveys near Daghabur to supplement earlier geological and geophysical surveys.

The ERTS imagery clearly shows the outcropping volcanics and Hamanlei Formation sediments in the Marda Range, the linear valleys of the Fafan, Gerer and Wabi Shebelli rivers, and numerous linears (Fig. 1) between 10 and 100 km long, several of which near Jijiga have been proved to be wrench faults⁵. Numerous NE–SW linears are also seen, which in the southern Ogaden seem to offset the zone. Similar cross-faulting has been mapped on the ground near Jijiga². Structural form lines determined from air photography consistently swing to the right near the zone (Fig. 2) suggesting dextral displacement on the faults.

South of Daghabur a positive Bouguer anomaly of $\sim 40 \times 10^{-5} \text{ m s}^{-2}$ trends NE–SW on the eastern side of the Marda lineament and east–west on the western side (Fig. 3). Similar Bouguer anomalies in the zone near Shilavo and Abred are associated with significant basement structure⁵, though the anomalies can only be adequately explained by assuming superposition of intrabasement and suprabasement density

contrasts as in the El Nala Bouguer anomaly in Saudi Arabia⁶. Profile analysis of the Daghabur anomaly defines a residual anomaly of almost $5 \times 10^{-5} \text{ m s}^{-2}$, which may indicate suprabasement structure. This is supported by a negative magnetic anomaly of $\sim 10^{-7} \text{ T}$ coincident with the gravity anomaly. The main anomaly may be interpreted in terms of crustal structure similar to the Bouguer gravity anomaly on the zone near Jijigga. In this case, major arching of the Moho discontinuity appears coincident with the Marda Fault zone⁷.

Black *et al.*³ defined a Tertiary age for the zone, evidenced by the abrupt contact of Mesozoic and Tertiary outcropping units across the zone, the displacement of Mesozoic sediments by NW-SE faults near Jijigga and the volcanics of the Marda Range which locally overlie the Cretaceous Amba Aradam sediments⁸. Mesozoic activity on the zone is, however, evidenced by structural thinning of

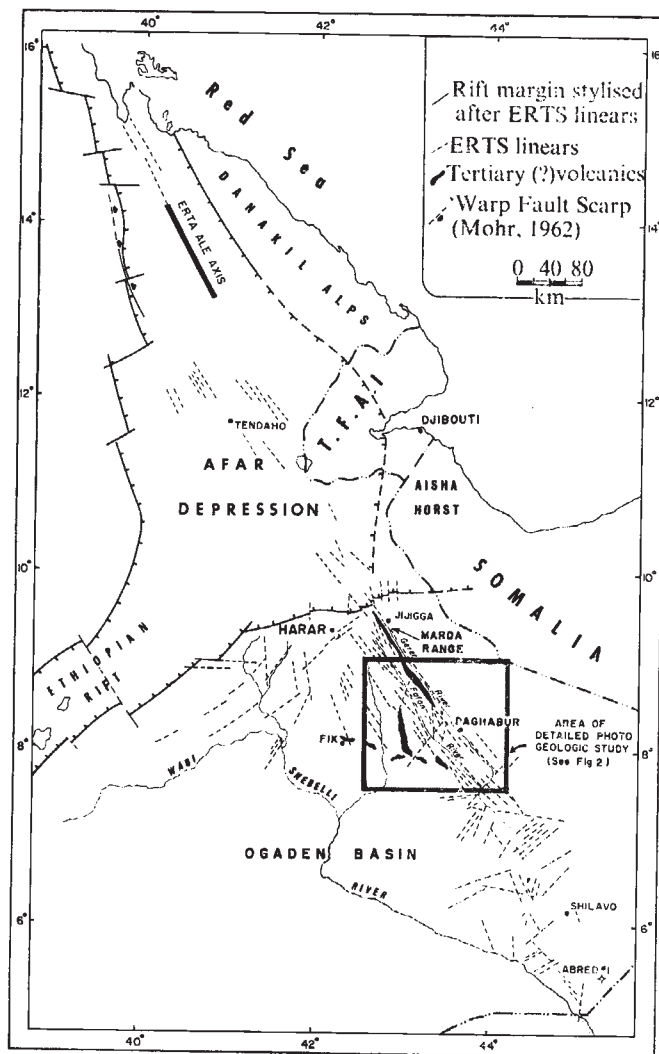


Fig. 1 Location map showing ERTS linears along the Marda Fault Zone. Only linears on trend with the zone are shown in the Afar Depression and most linears on the southern Somalia plateau have been omitted for clarity. 'Tertiary Volcanics' refers only to the volcanics on the Plateau in the vicinity of the Marda Fault.

the lower Jurassic Hamanlei Series on the basement structures, confirmed by reflection seismic surveys, and the thinning of Upper Jurassic limestones west of Jijigga⁹. The large Bouguer anomalies on the zone and the basic change in character of the regional Bouguer field across the zone have been interpreted as indirect evidence of Precambrian tectonism⁵. Mohr¹⁰ has noted that the NW-SE faulting near

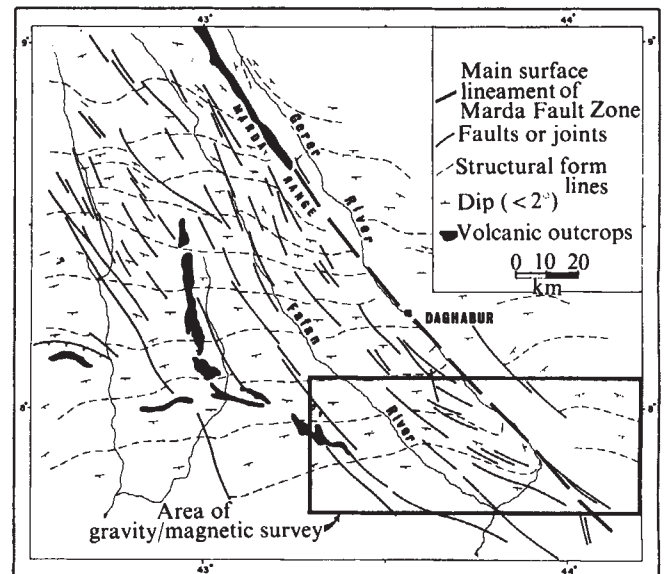


Fig. 2 Structural photogeology map of the Marda Fault Zone between $9^{\circ}00'N$ and $7^{\circ}40'N$. This interpretation is part of an unpublished company report by Photogravity Inc., Houston.

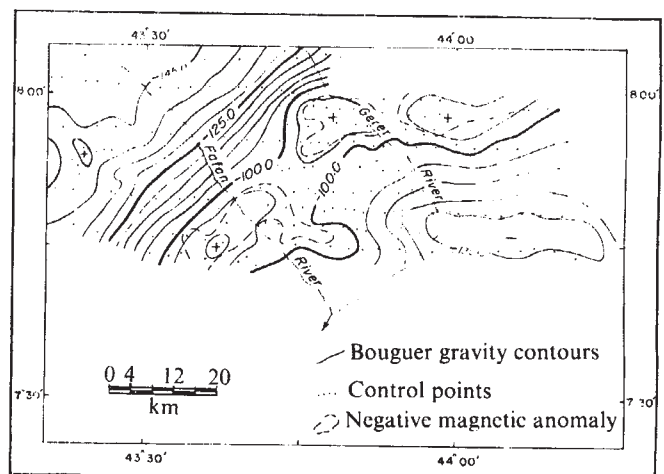
Tendaho, the alignment of Pleistocene volcanic centres on the Afar floor and the sharp reduction in the magnitude of the Afar margin structures east of the Marda Fault, point to the "continuing influence of the Marda line on Neogene tectonism of the southern margin of Afar despite the apparently extinct tectonism of the line itself". These indications of activity in the zone from Precambrian to Recent times qualify the proposal that the Marda Fault marks a zone of crustal weakness, continuing the Red Sea trend across the horn of Africa.

The fault zone has been projected via a line of epicentres into the Eritrean volcanic axis, possibly as part of a major mid-Tertiary shear zone extending along the Red Sea³. Alternatively, the fault has been projected north-westwards across the Afar into the highlands of Eritrea where it corresponds with a zone of Precambrian faulting and a significant facies change in the Jurassic limestones⁶.

Current detailed geological mapping of the zone southwards to $7^{\circ}50'N$ and projected additional gravity and magnetic surveys will hopefully provide new insights into the nature and age of the fault zone and its regional significance.

I thank the management of Whitestone International Inc., and L.L. & E. International Inc., for permission to comment on results of the companies' current exploration

Fig. 3 Bouguer gravity map of a portion of the Marda Fault Zone between $7^{\circ}30'N$ and $8^{\circ}10'N$.



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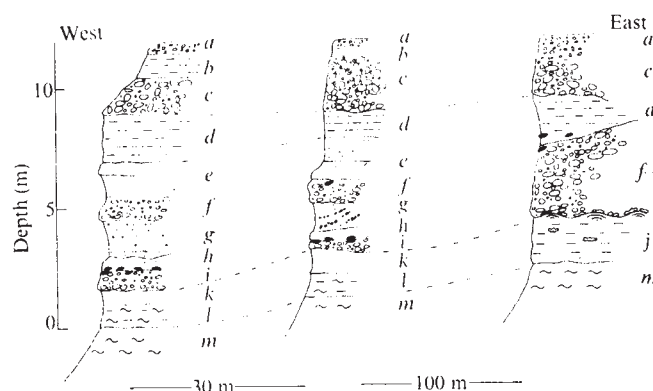


Fig. 1 East-west profile of site Hdr 56: a, residual gravel; b, thin layer of silt, disappearing to the east; c, boulder bed with occasional artefacts of the Hdr 10 type; d, brown, sandy silt; e, sands, partly cemented, partly loose; f, brown bouldery conglomerate, thinning out westwards; g, loose gravel and sand, disappearing eastwards; h, cemented sand with artefacts at the base; i, brown boulder conglomerate with artefacts at the top; j, grey, sandy silt with lime concretions and a lime crust at contacts with the boulder conglomerate; k, grey, loose sand with limonitic staining; l, grey, silty clay; m, brown clays of the Hadar Formation.

Prehistoric exploration at Hadar, Ethiopia

DURING the third International Afar Research Expedition, which led to the exciting find of the australopithecine skeleton known as "Lucy"¹, parallel investigations revealed the existence of two Early Stone Age (ESA) industries in the Hadar area. One comprises an Acheulian workshop (the Denen Dora Koma Acheulian), and the other, stratigraphically younger, constitutes a degenerate biface-flake industry.

The oldest known artefacts from the Hadar area (within the Afar Rift in Ethiopia) come from the Afaredo site and comprise a few basalt flakes from a silt within the upper part of the fluvio-lacustrine Hadar Formation. They have sharp edges and retain much of the cortex on their dorsal faces, besides one or two former flake scars. The calcareous, in part tuffaceous, silt occurs in an 8-m section of silts and sands on the bank of the Afaredo River and contains a small channel of tuff about 0.50 m thick. The flakes were embedded in the top of the silt at the contact with fine sand, constituting an ancient weathered horizon with small lime pellets. The silt is stratigraphically younger than the hominid levels but older than the Acheulian horizon capping the Hadar Formation. The time interval separating the hominids from the flake-bearing silt horizon seems to be shorter than the interval between the flake horizon and the Acheulian. Pending more detailed work, these findings remain too tenuous to allow a precise interpretation.

Following last year's exploration² of the Denen Dora Valley, it has been possible to map more than 120 artefacts on the surface of Holocene terraces in one of the small western tributary gullies of the Denen Dora. The concentration of tools increased upstream.

As the tributaries cut into the Hadar deposits precipitous slopes are formed in the area of Denen Dora Koma (mountain). At the top, lying unconformably over brown clays of the Hadar Formation, is a series of fluvatile

gravels, sands and silts, capped by a boulder bed about 60 m above the bed of the Denen Dora River. It is in this fluvatile series that the Acheulian horizon occurs, at the contact between a conglomerate and a cemented sand (Fig. 1). The section forms a vertical cliff about 10 m high, above the more gently sloping clays of the Hadar Formation.

The horizon seems to be extremely rich, and the whole slope was found to be covered with bifaces. The artefacts are fresh and sharp. Small flakes and chips with equally sharp edges are also present amongst the bifaces. Most probably the site was formerly an Acheulian factory. All the artefacts along the Denen Dora are derived from this site by erosion of the escarpment.

The Denen Dora Koma industry was evidently an Acheulian biface industry of an advanced character. Tools were found from the surface sites Hdr 9, 22, 23, 24, 25, + up, along the Denen Dora and *in situ* from sites Hdr 50, 55, 56 at the Denen Dora Koma. The assemblage comprises 226 artefacts collected in 1974, and 29 artefacts found a year earlier; 46 further artefacts from the same site derive from another industry (Hdr 10). Preliminary analysis of collected material reveals the tool classes shown in Table 1. Basalt, trachytic ignimbrites and rhyolites were used as raw materials. The assemblage is homogeneous in type and shape. No cleavers have been found; symmetrical, elongate-ovate forms with rounded points were evidently preferred for hand axes. They were made from flat and split pebbles. Most of the tools retain cortex near the butt or at the upper face. A few miniature forms with sizes of 7-10 cm have also been found.

Geological evidence indicates that the Denen Dora Koma Acheulian was situated at the edge of a flood plain. The

Table 1 Material from the Denen Dora Koma Acheulian

	Site (Hdr No.):													Total
	9*		22*		23*		24*	25	25 up	50	55	56	42	
Bifaces	5	4	15	1	2	2	1	20	39	16	1	54	8	168
Scrapers												2		2
Flakes	1	1	3					9	5	2	1	38	4	64
Cores	1				1			1	2	1		3	1	10
Modified pebbles								3	1			6	1	11
Total	7	5	18	1	3	2	1	33	47	19	2	103	14	255

*Collected during 1973-74; all others collected in 1974.

Table 2 Material from site Hdr 10

	Excavation						Surface			Total
	1973 Tr. I	Tr. II	1974 Tr. III	<i>in situ</i>	1973 10-I, -II	-III	10-I, -II	1974 -III	-IV	
Bifaces			9				1	1	4	15
Protobifaces		1	2	2	1	10	2			18
Choppers	1			1	2	4		2		10
Polyhedrons	1					1				2
Discoids						1				1
Modified pebbles			8			5				13
Flakes	2	2	31	4	7	5	3	7	2	63
Cortex flakes		1	9		1	3	1	2		17
Flake-like pieces						1				1
Cores		1	11	1	1	2				16
Total	4	5	70	8	12	32	7	12	6	156

stream deposited coarse gravel in the channel in swift water, and sands along the point bars, which were apparently occupied by man. The site became sealed by coarse fluvial gravel and later by over-bank deposits as the channel meandered further east. The site was probably occupied only temporarily, and the artefacts will have been quickly buried under frequent flood deposits. So far no associated bones have been found.

The Denen Dora Koma sequence is a local series of fluvial beds which crop out only at sites Hdr 50, 55 and 56, sandwiched between the Hadar Formation and a boulder bed. Otherwise the Hadar Formation is overlain directly and uncomfortably by the boulder bed. A trench (3×9 m), dug into the boulder bed at site Hdr 10-III (which last year yielded artefacts of an apparently pre-Acheulian industry), revealed 70 artefacts dispersed throughout the deposit at various random levels. They exhibit a crude technique of manufacture: cores are unprepared, and flakes in most cases retain some cortex at the dorsal face. No choppers like those from the surface have been found, though there are several modified pebbles. A few bifaces show Acheulian features but are crudely made, and others can only be described as proto-handaxes. Nonetheless, the few bifaces recovered from the excavation indicate a younger age than previously suspected, especially as the Denen Dora Koma Acheulian occurs in a stratigraphically older horizon beneath the boulder bed.

The tools in the assemblage found at site Hdr 10 (Table 2) are characterised by minimal workmanship. The industry seems to have produced tools which could be used for certain types of work, with a minimum of flaking. The industry does not look as if it was heterogeneous and does not seem to have been transported from different sources.

Several nearby localities have yielded artefacts of the same type, all derived from the boulder bed: sites Hdr 33, 36, 37, 38 and 39 are surface sites on the terraces along the Sidi Hakoma River; sites Hdr 46, 47, 48, 49 and 53 are all from the boulder bed in the vicinity of Hdr 10, a little to the north-west. Site Hdr 62 is from the boulder bed between the Sidi Hakoma and the Afaredo rivers.

The industry at site Hdr 10 can be called a degenerate biface-flake industry, operated by a different and somewhat later group of people than the makers of the Denen Dora Koma Acheulian. They neglected the Acheulian tradition, or imitated it crudely. A rather similar phenomenon can be seen at Olduvai in Bed IV, where a true Acheulian culture is apparently more or less contemporaneous with that of a developed Oldowan with degenerate bifaces (M. D. Leakey, unpublished).

It is interesting that in the Hadar area evidence of a degenerate biface-flake industry exists in the boulder bed stratigraphically above the advanced Acheulian biface assemblage which occurs in the underlying fluvial sands. Both are *in situ* and are in juxtaposition at site Hdr 56. The boulder bed, lying unconformably over the Hadar

Formation, is therefore younger than previously assumed, correlating with Bed IV at Olduvai rather than with Bed II.

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Australopithecus, Homo erectus and the single species hypothesis

AN enormous wealth of early hominid remains has been discovered over the past few years by expeditions within eastern Africa. Evidence has been presented for the existence over a considerable period of time of at least two contemporaneous hominid species¹. Some of this evidence is compelling, but some less so for a variety of reasons such as the lack of association, fragmentary specimens, geological uncertainties, equivocal anatomical differences and suchlike. Many of these new specimens are of great antiquity and have led to suggestions that an early form of the genus *Homo* was contemporary with at least one species of *Australopithecus*. The evidence presented here deals not with the earlier stages of human evolution, but with the unequivocal occurrence of *H. erectus* from the Koobi Fora Formation, east of Lake Turkana (formerly Lake Rudolf).

Among the variety of hypotheses put forward to accommodate the evidence in an evolutionary framework, the most explicit and directly simple is the single species hypothesis². This hypothesis rests on the assumption that dependence upon tools was the primary hominid adaptation that enabled expansion into open country environments. In the clearest exposition of the hypothesis³, basic hominid characteristics, including bipedal locomotion, reduced canines and delayed physical maturity are seen to have come about in response to more effective and greater dependence on culture. This assumption of a basic hominid cultural adaptation allied with the principle of competitive exclusion^{4,5} leads to the conclusion that two or more hominid species would be extremely unlikely to exist sympatrically. Here we present decisive evidence that shows the existence of two contemporaneous hominid species in the Koobi Fora area.

The adult cranium KNM-ER 3733 (ref. 6) was found *in situ* in the upper member of the Koobi Fora Formation⁷. The sediments lie stratigraphically between the KBS and the Koobi Fora/BBS tuff complexes. The cranium consists of a complete calvaria and a great deal of the facial skeleton, including the nasal and zygomatic bones. The

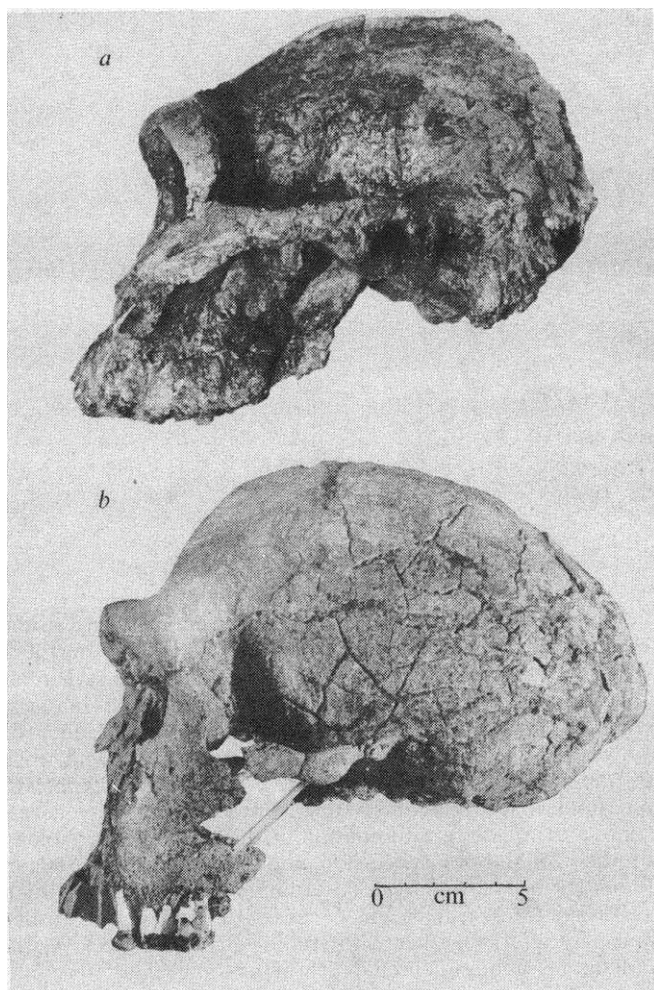


Fig. 1 Lateral aspect of KNM-ER 406 (a) and KNM-ER 3733 (b).

nearly complete alveoli of the anterior teeth and examples of the premolars and molars are preserved. The third molars were lost before fossilisation. Preparation has been limited so far to the external cranial surfaces and part of the brain case. An endocranial capacity is unknown, but by comparison is likely to be of the order of 800–900 ml. In all its features the cranium is strikingly like that of *H. erectus* from Peking⁸. Such orthodox anthropometric comparisons that can be made at present fall well within the range of the Peking specimens.

The cranium is large (glabella to inion/opisthocranium is 183 mm) with large projecting supraorbital tori and little postorbital constriction (minimum postorbital breadth

91 mm). There is a marked postglabellar sulcus and the frontal squama rises steeply from behind it to reach vertex at bregma. The skull decreases in height from bregma and the occipital bears a pronounced torus where the occipital and nuchal planes are sharply angled. The greatest breadth is low at the angular torus (biauricular breadth 132 mm). There are strong temporal lines that are 60 mm apart at their nearest point. As far as can be judged at present, the vault bones are thick (about 10.0 mm in mid-parietal). The temporal fossae are small. The facial skeleton is partly preserved and shows deep and wide zygomatic portions, longitudinally concave and laterally convex projecting nasals and wide, low piriform aperture. The incisive alveolar plane is short and wide and the large incisive alveoli are set almost in a straight line. There are strong canine juga. The palate is high and roughly square in outline. The facial skeleton is flexed under the calvaria and in the preliminary reconstruction is set at about the same angle as that reconstructed for a female *H. erectus* by Weidenreich⁹. The prosthion to nasion length is 87 mm. This is the best preserved single *H. erectus* cranium known, the facial skeleton being more complete and less distorted than that of 'Pithecanthropus VIII' (ref. 9).

Figures 1–3 show KNM-ER 3733 together with KNM-ER 406, another cranium discovered *in situ* in the Upper Member of the Koobi Fora Formation. This latter specimen has been described¹⁰ in some detail and is clearly that of a robust *Australopithecus*. Other specimens of robust *Australopithecus* have been found in deposits of both the Upper and Lower members of the Koobi Fora Formation. Two *in situ* mandibles that must have been associated with this type of cranium are the most massive representatives of this species. KNM-ER 729 (ref. 1) was excavated in 1971 from the base of the Middle Tuff and KNM-ER 3230 (ref. 6) from the upper part of the BBS complex. Other, more incomplete, specimens of robust *Australopithecus* mandibles have come from the higher levels of the Formation between the Karari/Chari and Koobi Fora/BBS tuffs. The radioisotopic dating of these tuffs, in spite of a continuing controversy over one of them¹¹, is not an issue here. The contemporaneity of *Homo erectus* and a robust *Australopithecus* is now clearly established over the period during which the Upper Member of the Koobi Fora Formation was deposited. Using the time scale given by Fitch *et al.*¹², this would be from earlier than about 1.3 to earlier than 1.6 Myr ago.

The new data show that the simplest hypothesis concerning early human evolution is incorrect and that more complex models must be devised. The single species hypothesis has served a useful purpose in focusing attention on variability among the early hominids and also on the ecological consequences of hominid adaptations. Alternative concepts, especially those concerning niche divergence and

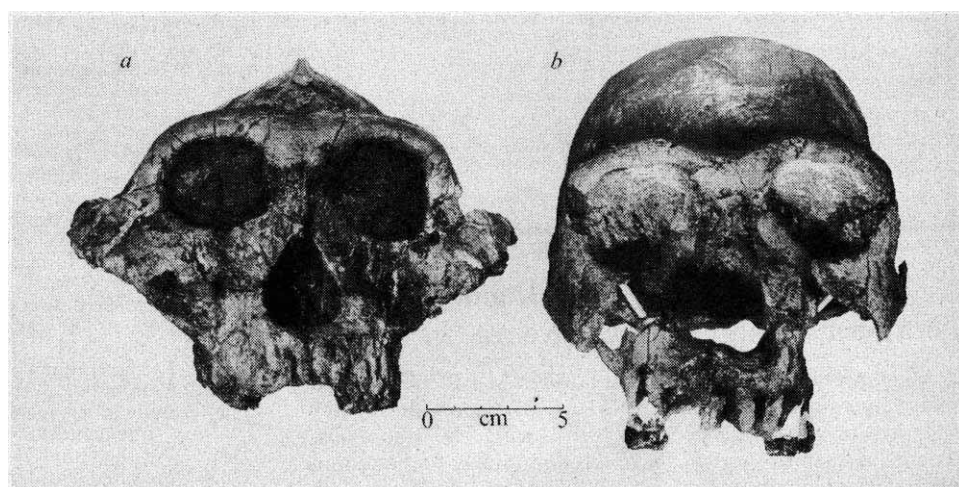


Fig. 2 Frontal aspect of KNM-ER 406 (a) and KNM-ER 3733 (b).

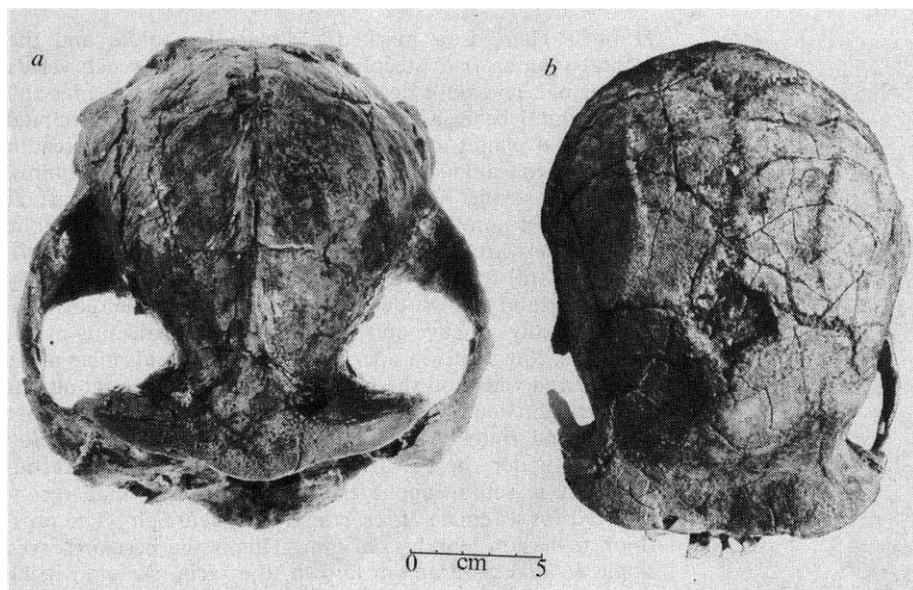


Fig. 3 Superior aspect of KNM-ER 406 (a) and KNM-ER 3733 (b).

sympatry, should now be formulated. We think that populations antecedent to *H. erectus* ones have been sampled in the Koobi Fora Formation (specimens include KNM-ER 1470, 1590 and 3732). The clear demonstration of at least two hominid species earlier in time should enable us to reconsider our approaches to the problems of earlier hominid evolution. We also think that there is no good evidence for the presence of *Australopithecus* outside Africa, and the finding of an apparently advanced *H. erectus* cranium at Koobi Fora provokes issues such as why *Australopithecus* is only an African form, the nature of its extinction and the apparent stability of some hominid morphologies over long periods of time.

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field programme in the Koobi Fora Formation, lying to the east of Lake Turkana (formerly Lake Rudolf) in northern Kenya. (This area was previously known as East Rudolf and has been studied since 1968; ref. 6.) The material reported here has been referred to either the Upper or Lower Member of the Koobi Fora Formation. Recent work on the dating of tuffs by Curtis *et al.*⁷ has raised important questions that will require time to resolve and may necessitate a complete re-examination of the reported chronology of the Koobi Fora succession and all other East African Plio-Pleistocene localities. Alternative dating methods such as fission track will be given close attention in the future and may offer, together with studies of certain taxa, useful indications about the reliability of isotopic age determinations.

The right innominate KNM-ER 3228 (Fig. 1) was discovered by Bw. Bernard Ngenao in area 102 in the Lower Member. The specimen was found encrusted with a well

Table 1 Hominid remains from East Rudolf, 1974–75

KNM nos	Specimen detail	Area	Stratigraphic position (Member)
2592	Parietal fragment	6	Ileret
2593	Molar fragment	6	Ileret
2594	Proximal fragment of tibia and fragment of tibia shaft	6A	Ileret
2595	Cranial fragments	1A	Ileret
2596	Distal fragment of tibia	15	Upper
2597	Molar crown	15	Lower
2598	Occipital fragment	15	Lower
2599	Fragment of premolar crown	15	Lower
2600	Partial molar crown	130	Lower
2601	Molar	130	Lower
2602	Cranial fragments	117	Lower
2603	Tooth fragment	117	Lower
2604	Tooth fragment	117	Lower
2605	Tooth fragment	117	Lower
2606	Tooth fragment	117	Lower
2607	Tooth fragment	105	Upper
3228	Right innominate	10	Lower
3227	Mandible with both P ₃ crowns	103	Upper
3230	Mandible with full dentition	131	Upper
3728	Shaft and neck of right femur	100	? Lower
3729	Body of left mandible, partial roots C–M ₃	102	? Upper
3730	Weathered distal femur	102	? Upper
3731	Body of left mandible, partial roots I–M ₃	105	Lower
3732	Partial cranium	105	Lower
3733	Cranium	104	Upper
3734	Body of left mandible, C–M ₂ , fragment M ₃	105	Lower
3735	Weathered distal right humerus	116	Lower
3736	Proximal two-thirds of a radius	105	Upper

New hominid fossils from the Koobi Fora formation in Northern Kenya

A WELL preserved partial innominate KNM-ER 3228, and a relatively complete cranium KNM-ER 3733 provide further support for the presence of *Homo* during the Plio-Pleistocene in eastern Africa^{1–5}. These hominid specimens and others (Table 1) were collected during the 1974 and 1975

cemented matrix containing small pebbles and shells. The specimen seems to have eroded from a fluvial lens within a generally lacustrine section. The presence of similar pebbles and shells in this lens has been established.

The specimen is large, robust and from an adult male. The pubic and ischial rami are missing, having been broken away at the ilio-pectineal eminence at the inferior part of the tuberosity of the ischium. The acetabulum is large and it has been damaged along the pubic part of the rim. The position and orientation of the ischial tuberosity is similar to that observed in a sample of modern *H. sapiens*, as are a number of other morphological characteristics. Certain features such as the marked and thick acetabulo-cristal buttress are similar to those reported by Day⁸ on the Olduvai innominate OH 28, and there are few resemblances to the material referred to as *Australopithecus*⁹. The apparent lateral flare of the ilium is accentuated by the acetabulo-cristal buttress and the marked concavity of the postero-lateral region of the iliac blade. Measurements are given in Table 2 and a detailed description will be presented elsewhere.

The cranial fragment KNM-ER 3732, discovered *in situ* but extensively weathered, includes the major part of the left orbit, the frontal region and the top of the vault, which is broken away near the squamous and lambdoidal sutures. The specimen is fractionally smaller, but strikingly similar to KNM-ER 1470 (refs 10 and 11) and thus provides a further example of this pattern of cranial morphology in the early hominids.

The cranium KNM-ER 3733 (ref. 12) was discovered by Bw. Ngeneo in area 104 in deposits of the Upper Member. The specimen was *in situ*, the supraorbital tori being just exposed on the surface. The maxillary and facial skeleton was fragmented and exposed within a distance of about 50 cm from the vault. Tooth crowns and an assortment of small fragments were recovered by sieving the area immediately below the skull on the slope. The cranium is large with only a slight postorbital constriction. The supraorbital tori are prominent, protruding anteriorly. The specimen is very similar to the *H. erectus* material from China (R. E. F. L. and A. C. Walker, unpublished) and is therefore assigned to *H. erectus*.

The archaeological research group which works under the direction of Glynn Isaac, recovered a hominid fossil from one of its trenches. The specimen, KNM-ER 3230 (Fig. 2) came from the site FxJj 20 (east) which was being excavated by J. W. K. Harris as a part of his intensive study of the archaeology of the Upper Member. The fossil is a mandible with a complete dentition (Table 3), but unfortunately the rami are not preserved. It is a typical example of the robust *Australopithecus* of which numerous specimens have previously been reported from East Africa^{1-5, 13-16}. The mandible was found by Bw. Kitibi Kimeu while consolidated brown silts were being dug away to uncover a major archaeological occupation floor which had been identified previously. It lay approximately 15 cm above the main artefact-bearing horizon, and there were a few scattered flakes at the same level (J. W. K. Harris, personal communication). The circumstances do not necessarily constitute evidence of a significant connection between the particular hominid and a stone tool industry, since there are discarded artefacts scattered throughout the Upper Member silts over very wide areas.

The left femur KNM-ER 3728 was discovered *in situ* by Bw. Maundu Muluila in Area 100. The specimen has suffered extensive abrasion on the proximal and distal ends with the condyles, lesser and greater trochanter and head being lost. The marked antero-posterior compression of the long neck compares well with femora that have been assigned to *Australopithecus*¹⁷, but the long slender platymeric shaft is unexpected and provides intriguing evidence for the possible variation of the femur in this genus.

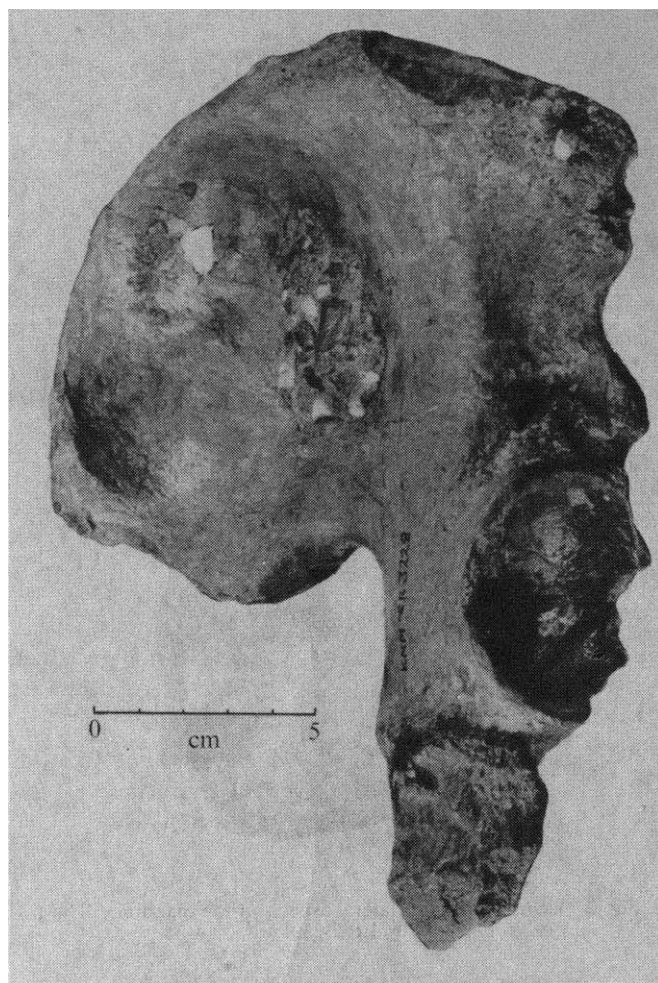


Fig. 1 Lateral aspect of the right innominate, KNM-ER 3228.

Among the new collection, the innominate is of particular interest and it can be clearly distinguished from the fossil innominates reported from Sterkfontein and Swartkrans.

Similarities to OH 28 from Olduvai Gorge and the essentially modern appearance of this fossil require that it be attributed to *Homo*. Robinson¹⁸ proposed that the large acetabulum and the well developed acetabulo-cristal buttress were evidence that OH 28 was aberrant and that the Swartkrans fossils, such as SK 3155(b) be considered as examples of transitional *Homo* innominate morphology. He suggested a very late development of the modern hip in which the loading per unit area of articular surface is reduced. The new specimen requires that this argument be re-examined.

Similarly, the proposal by Wolpoff and Lovejoy¹⁹ and others for a single pattern of pelvic morphology cannot be maintained. Although their observations on the mechanical

Table 2 Measurements (mm) of the innominate bone KNM-ER 3228

Anterior superior iliac spine to posterior superior iliac spine	166
Anterior superior iliac spine to posterior inferior iliac spine	147
Anterior superior iliac spine to deepest part of greater sciatic notch	115
Anterior superior iliac spine to centre of acetabulum	110
Posterior inferior iliac spine to superior margin of acetabulum	105
Diameter of acetabulum (superio-inferior)	57
Thickness of acetabulo-cristal buttress just above acetabulum	25

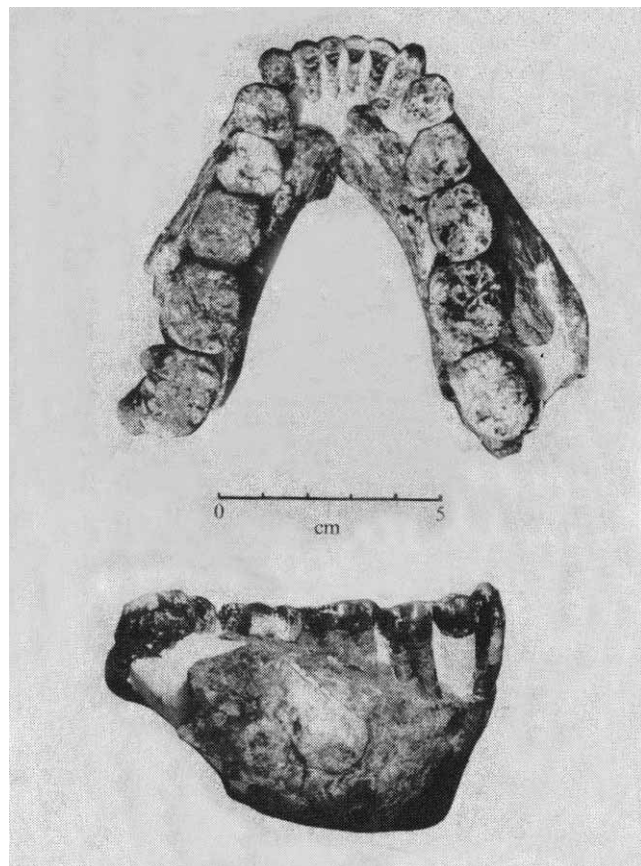


Fig. 2 Occlusal and right lateral aspects of the mandible, KNM-ER 3230.

issues involved in small brained, bipedal hominids may be correct, there is evidence for a second model which presumably reflects the mechanical requirements of a large brained, striding biped. The new innominate together with femora such as KNM-ER 1481 (refs 10 and 11) provide critical evidence for early modification of the hip joint in *Homo* and this adds weight to the argument for the late Pliocene separation of *Homo* and *Australopithecus*.

The East African fossil evidence for the Pliocene differentiation of *Homo* is still considered by some to be tenuous, but I am impressed by the cranial and postcranial data that are now available. There is an obvious need for more precise differential diagnoses. The latest attempt¹⁹ is unsatisfactory because it is apparently based on an hypothetical model which is now invalidated by new empirical evidence. From early Pleistocene levels, and later, there does seem to be good evidence for the *Homo* lineage, and the principal specimens would include KNM-ER 1470 (ref. 10), 1590 (ref. 4), 1481 (ref. 10), the new innominate 3228 and the cranium KNM-ER 3733. The larger endocranial volume (upwards of 700 cm³) and the large anterior dentition as well as the hip distinguish this group from other hominids.

Table 3 Measurements (mm) on KNM-ER 3230

	Left side		Right side	
	md	bl	md	bl
I ₁	5.1	6.5	5.1	6.2
I ₂	6.9	—	6.3	8.0
C	7.8	9.2	7.5	9.1
P ₃	11.5	14.6	11.0	14.1
P ₄	13.8	—	14.0	16.9
M ₁	(17.0)	—	16.6	15.4
M ₂	(20.0)	—	20.1	18.5
M ₃	20.5	16.2	21.2	16.9

The question of species distinction within *Australopithecus* is problematical, but I am of the opinion that two contemporary species can be defined on the evidence now available. The most obvious is the robust form *A. boisei*²⁰ and this is well represented from late Pliocene horizons and continues until well into the Pleistocene (about 1 Myr). There may be some merit in treating this material as a subspecies of *A. robustus*. The evidence for a second species in East Africa is less convincing at present but there are specimens such as KNM-ER 1813 (ref. 4) that have the small endocranial volume (less than 550 cm³) and generally gracile characteristics that seem to distinguish them from the robust species. One of the most distinctive features is the small size of the cheek teeth and small anterior teeth. Note also that at present, there is no convincing evidence for *Australopithecus* occurring earlier than examples of the *Homo* lineage. Certain specimens such as the Lothagam mandible have been attributed to *Australopithecus*²¹, but the fragmentary nature of these specimens and the lack of diagnostic features would suggest that even generic attribution is not possible at the present time. Such specimens are more correctly referred to Hominidae indet.

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World's oldest animal traces

THE discovery of animal burrows in the basal beds of the main ore horizon on the Zambian Copperbelt provides evidence for the existence of metazoan life some 300 Myr older than the previously oldest known fauna, the late Proterozoic Ediacara fauna¹. I describe here two kinds of burrow (referred to as types A and B pending more formal nomenclature), their geological setting, and age relationships.

Type A burrows are complex (Fig. 1); isolate forms are rare and multiple burrowing along narrow stringers (2-5 cm) is common. Complete bioturbation extending over several centimetres has been noticed at three horizons (Figs 2 and 3). The burrow walls are laminated parallel to the long axis of the burrow, and the infill exhibits a cusped ('spreite') structure (Fig. 1), probably indicating active back-filling by the animal responsible. Branching of an irregular frequency is common, a simple Y shape predominating with no size change taking place. The burrows range from 3 to 8 mm in diameter and 3 to 10 cm in length, though



Fig. 1 Complex type A burrows. Infilling shows spreite structure (arrowed) and laminated walls. Colour contrast with the surrounding rock reflects the differential reaction to tropical weathering of the chemical and mineral changes induced during the passage of sediment through the animal's gut⁶. Mindola North Pit, Kitwe, Zambia. Scale bar, 1 cm.

burrows up to 20 cm long have been noted. No preferred orientation has been observed (Fig. 2) and the burrows lie horizontally with respect to the bedding. Occasional forms lying obliquely to the bedding have been observed, though these are rarely more than 1–2 cm long.

Fig. 2 Bioturbation showing type A burrows. Note lack of orientation. Mindola North Pit, Kitwe, Zambia. Scale bar, 1 cm.



Type B burrows are simple, usually isolate forms. (Fig. 4). The wall structures have been masked by secondary replacement and accretion, but it is evident that the burrows were lined and not actively filled. There is evidence of partial filling, attributable to diagenetic secondary minerals and to wall collapse which allowed sediment influx. Branching is common, varying from simple Y shape to dendritic. The frequency is irregular, and a size reduction after branching is apparent (Fig. 4). Diameters range from 2 to 10 mm with larger burrows frequently flattened to an ovate section. Continuous burrows, greater than 1 m in length have been observed (Fig. 4). The burrows lie horizontally relative to the bedding and no preferred orientation has been observed.

The sediments containing the burrows are the two basal units of the main ore horizon in the Roan Group of the Katangan Complex in Zambia (Table 1 and Fig. 3). The sediments represent the basal, littoral facies of a marine transgression which occurred after an extensive period of

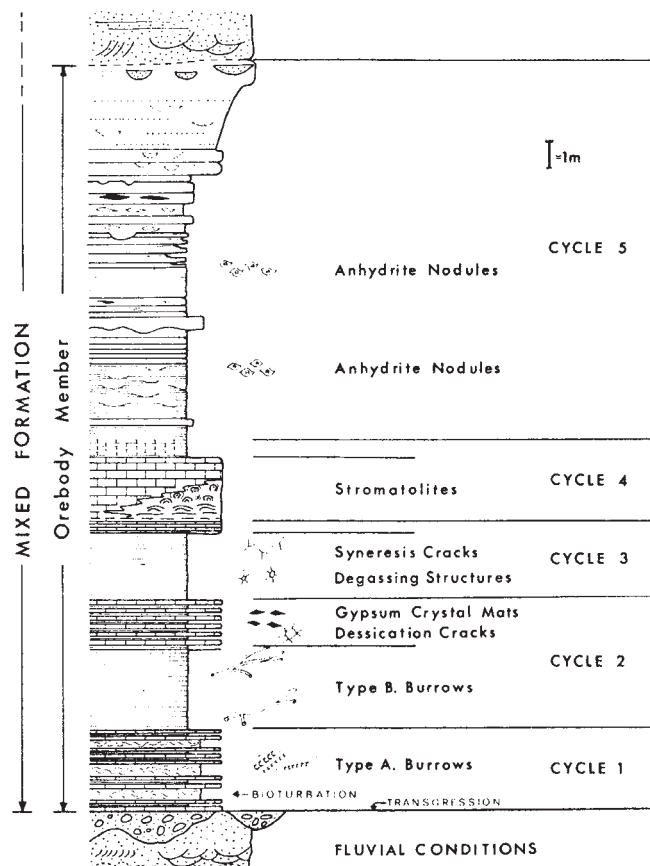


Fig. 3 Log of the containing sediment at the type locality. Each cycle consists of a deepening ('sub-tidal') and shallowing ('intertidal') facies. Cycle 1 is incomplete as only the 'intertidal' unit of the transgression is present. Mindola North Pit, Kitwe, Zambia.

continentality². During the early stages of the transgression the sea seems to have had intermittent connections with the main ocean, and the energy of the environment remained low until cycle 4 (Fig. 3). The unit overlying the burrowed horizons (top of cycle 2 on Fig. 3) displays evidence of highly saline conditions during deposition, and of reducing conditions in the sediment. Both of these factors would preclude the existence of an indigenous fauna, and it is notable that burrows are absent from this horizon.

A minimum age for the containing sediment is set by dates on syntectonic pegmatites from basal Roan Group rocks, of 888 ± 42 Myr BP and 840 ± 40 Myr BP (ref. 3). Combined lithostratigraphic, biostratigraphic (stromatolites), and chronostratigraphic studies have established an age of

Table 1 Stratigraphy and approximate time range of the Katangan in Zambia and Zaire

COMPLEX	GROUP	FORMATION	Age (Myr)
KATANGAN	Kundelungu	Upper	620
		Middle	680
		Petit Conglomerate	800
		Lower	950
	Mwashia	Grand Conglomerate	
		Dolomite	
	Roan	Mixed	
		Burrows	1050
		Coarse Clastics	

1,000–1,050 Myr BP for the main ore horizon in the Roan Group of Southern Shaba in Zaire, and indicate that this horizon correlates with the main ore horizon in the Roan of Zambia; recognition of the depositional cycles depicted in Fig. 3 in the ore horizon of Komoto in Shaba (unpublished) confirms this. Thus an age of around 1,000 Myr for the burrows is reasonable.

The date of 1,000 Myr shows the burrows to be considerably older than the Ediacara fauna which has a time range of 600–700 Myr BP at 10 localities around the globe¹. According to Glaessner⁴ the oldest known evidence for animal life, the trail *Burerichnus*, is found in the Brachina Formation some 1,500 m below the base of the Cambrian but 300 m above the uppermost glacials, the Eltina Group of the Adelaidean System in the Flinders Range of Southern Australia. The Marianoan Tillites of the Eltina equate with the Petit Conglomerate, uppermost of two

tillites in the Katangan. The Sturtian Tillite of the lowermost Adelaidean glacials equates with the Grand Conglomerate, the lowermost of the Katangan Tillites (Table 1). The burrows reported here are at least 1,000 m below the Grand Conglomerate.

The question remains of why, after being at such an advanced stage of evolution in the basal Roan sediments, the animals did not continue to flourish, for the overlying sediments are devoid of animal fauna. The answer may be that the fauna was abandoned after encroaching on to the continent during the first transgression. The disappearance of burrows from the sedimentary column during the deposition of the highly saline beds at the top of cycle 2 on Fig. 3 could be a reflection of this.

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Explanation of large scale extinctions of lower vertebrates

A MAJOR area of speculation in palaeobiology is the search for the “causes” of massive cross-group extinctions such as that at the end of the Cretaceous. A controversial recent addition to the literature on the phenomenon of extinction itself is the work of Van Valen¹, who attempts to show that extinctions are stochastically constant over time for given groups of organisms. Lacking from Van Valen’s study of group survivorship, however, is consideration of the history of diversification within each group. In an empirical study I have attempted to demonstrate features common to the diversifications of lower vertebrates and other organisms.

Figures 1 and 2 show the course of diversification of groups of lower vertebrates and certain invertebrates. For each group the numerical diversity (genera) at any given time is plotted as a percentage of maximum diversity observed for any similar time interval (the subdivision of the time axis, and thus the precision of the plots, varies according to the level of information available in compilations from the fossil record^{2–4}). At the ordinal or subordinal level of traditional classifications of lower vertebrates a simple curve of diversity can be identified with a single major peak for each group; higher categories give multiple peaks. Although groups may persist in the fossil record for long times before the peak diversification, the curves for all groups are essentially similar between 25% points on the ascending and descending arms of the curve. The ascending slope of the curve approaches an exponential rather than a logistical growth pattern. The descending slope of each curve is usually quite symmetrical with the ascending curve, leaving only a short time span between 25% points on the curves. There are no major square-topped curves and the basic pattern is the same for groups diversifying at all times through the record. No group can be found that survives in the fossil record for significant lengths of time at or even near its peak diversity. Rapid extinction is thus a normal and immediate consequence of diversification for the groups shown.

This pattern is common to all groups of fishes, amphibians and reptiles for which adequate data are available⁴. It is also common to the following groups of invertebrates:

Fig. 4 Type B burrow. Note the simple form and size reduction on branching (arrowed). Mindola North Pit, Kitwe, Zambia. Scale in cm.



graptolites, cystoids, trilobites, nautiloids, Palaeozoic brachiopods, corals and ammonoids and possibly the ostracods. The following invertebrate groups (for which good data are available) do not show such rapid waxing and waning of diversity: bivalves, echinoids, gastropods. It is interesting that these last named seem relatively unaffected at the time of major extinctions of other groups³.

The common shape of the plots of diversity is readily explicable in terms of existing theory^{5,6}. The basic characteristics defining a particular group are in evidence (that is, the group can be recognised) before the main diversification begins. The main diversification then occurs largely at lower taxonomic levels through production of large numbers of specialised lower taxa within a given adaptive zone. An analogy can be made between this situation and the phenomenon of "species packing" and consequent niche reduction⁷. Regardless of the absolute number of taxa produced in each diversification, specialisation of taxa continues until a point at which normal environmental instabilities will make large numbers of taxa extinct. Hence the sharp decline in diversity after the peak. The specific components of the environmental instability may not be identifiable, but over geological time it is essentially a fundamental property of increased specialisation of taxa that some instability or other will cause extinction. Once diversification is entrained therefore (a process recognisable in hindsight as represented by approximately the 25% point on the ascending scale of the diversity curve) the rest of the process of diversification and extinction is predictable.

In addition to the shape of the "group diversity curves" the plots in Figs 1 and 2 show that the peaks in diversity of

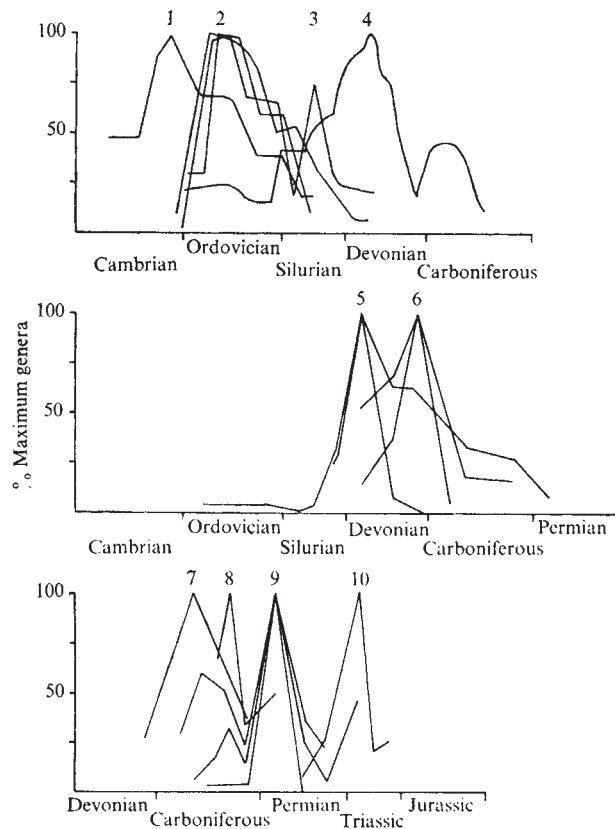


Fig. 1 Group diversity curves for the Palaeozoic. The peak diversities of different groups are identified by number: 1, trilobites; 2, cystoids, graptolites, nautiloids; 3, nautiloids; 4, brachiopods; 5, agnathan and acanthodian fishes; 6, placoderm and sarcopterygian fishes; 7, chondrosteian fishes and microsaurs; 8, nectridean amphibians; 9, rhachitome and anthracosaur amphibians; 10, stereospondyl amphibians.

disparate groups tend to be coincident in time, rather than dispersed randomly throughout the record. This distribution of peaks seems to show a 62-Myr periodicity with less consistent 31-Myr intervals⁴.

Comparison of Figs 1 and 2 shows that there is nothing different in the course of diversification and eventual extinction between groups going extinct in the Upper Devonian or Upper Cretaceous and any other groups. The major feature of the Permian is the lack of large numbers of groups diversifying in the Middle and Upper intervals. Permian vertebrates follow the common pattern. The Upper Devonian and Upper Cretaceous are remarkable, however, for the large number of groups diversifying more or less in synchrony.

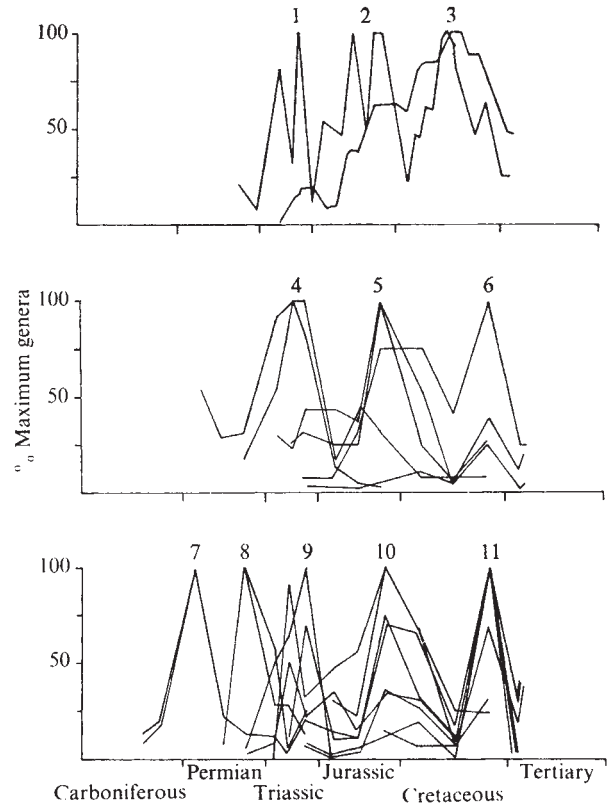


Fig. 2 Group diversity curves for the late Palaeozoic and Mesozoic. The peak diversities of different groups are identified by number: 1, ammonoids; 2, ammonoids; 3, ammonoids and corals; 4, palaeonisciform and semionotiform fishes; 5, amiiform and pholidophoriform fishes; 6, pycnodont and teleost fishes; 7, cotylosaur and pelycosaur reptiles; 8, cotylosaur and eosaurian reptiles; 9, ichthyosaur, thecodont and saurischian reptiles; 10, pterosaur, ichthyosaur, saurischian and crocodilian reptiles; 11, chelonian, crocodilian, saurischian, ornithischian, sauropterygian and squamatan reptiles.

If it is true that rapid extinction is inevitable we can refocus the search for causes. The important question about late Devonian and Cretaceous times (at least for the groups discussed here) is not why extinction occurred, but why so many groups diversified in synchrony at certain times in the Phanerozoic. It is necessary to find the major features of the physical and biological world common to these groups during their diversifications⁸⁻¹¹, rather than to find the signs of their minor perturbations that hasten their extinctions. Permo-Triassic extinctions of many invertebrate groups, however, still present a separate problem because there are no surges in diversity immediately preceding extinction.

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Visual attention in split-brain monkeys

How the separated hemispheres of a split-brain animal avoid conflict with each other remains uncertain. In some circumstances both hemispheres seem to be able to attend to separate stimuli simultaneously^{1,2}. Yet, when a split-brain animal has received separate training in each hemisphere on visual discrimination tasks which require opposite solutions, and is then placed in a situation where either hemisphere could respond, conflict does not occur. Instead, one hemisphere dominates behaviour for a variable time, then the other assumes control³. Independent variation of degree of attention in each hemisphere has been hypothesised to account for this⁴. We have sought electroencephalographic evidence of such hemispheric independence of visual attention in the split-brain animal, since a change in the power spectrum of the EEG accompanies increased visual attention—that is, an increase in θ (4-7 Hz) and β (13-25 Hz) frequency power, and a decrease in α (8-12 Hz) frequency power⁵. Power spectrum analysis has given evidence of alternate dominance of visual attention in the separated hemispheres. This finding has implications for the absence of hemispheric conflict in such animals, and the development of hemispheric specialisation in man.

Our subjects were three rhesus monkeys, which underwent surgical section of the telencephalic and tectal commissures, the optic chiasma, and in one case, the massa intermedia. During recording sessions the animals were seated in a restraint chair, allowed to gaze about them, but not required to make operant responses. Electroencephalograms from each occipito-parietal area were recorded on f.m. tape, and later computer analysed to give average power spectra over successive 2-min epochs. Variations in the power spectra of each hemisphere were compared with those of its partner.

Usually the power spectra from each hemisphere covaried with the animals' general state of arousal and in those conditions the difference between successive 2-min spectra had a strong tendency to be closely similar in the two hemispheres. A relative increase in power in the θ and β bands was associated with a fall in the α band. On some occasions, however, the difference spectra in the two hemispheres would become almost mirror images suggesting a shift in the dominance of attention from both to one or other of the hemispheres. To determine whether these spectral shifts, which seemed to occur spontaneously, were related to the degree of visual processing in each hemisphere, we then checked the effect of covering each eye of the subject alternately, thus restricting visual input to one hemisphere at a time. Now difference spectra that were mirror images could invariably be produced by changing the cover from one eye to the other every 2 min (Fig. 1).

These observations suggest independent variations in the degree of visual attention in each hemisphere of the split-brain monkey. It seems that an increase in visual attention in one hemisphere may occur at the same time as a decrease in attention in the other hemisphere. Such a phenomenon perhaps involves a "switching" mechanism located at the midbrain level, or below, since in one case the midline bisection included the massa intermedia, as well as the tectal and telencephalic commissures, optic chiasma and associated supraoptic commissures.

A mechanism of this type would permit one hemisphere or the other to dominate behaviour in the presence of stimuli which might otherwise result in interhemispheric conflict. The factors determining which hemisphere achieves dominance at any given time remain unclear.

In the special case of split-brain humans, asymmetries of the EEG between hemispheres during performance of verbal or spatial tasks have been described⁶. A neural mechanism controlling hemispheric dominance of attention could be an important influence leading to hemispheric specialisation in man.

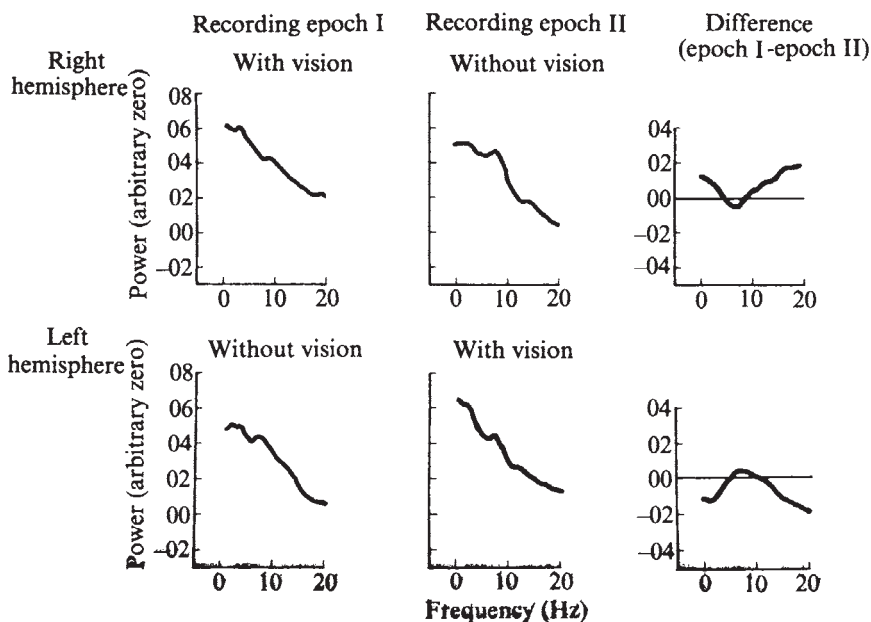


Fig. 1 The effect on the EEG power spectra of changing the cover from one eye to the other every 2 min. Difference spectra for the two hemispheres are mirror images, a relative increase in power in θ (< 7 Hz) and β (> 13 Hz) is associated with a fall in α (8-12 Hz) for the hemisphere visually attending. These results represent the average from three split-brain rhesus monkeys.

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Tubulin synthesis in developing rat visual cortex

In some species, restriction of sensory experience during early postnatal life can affect functional connectivity within the brain¹. Yet, in these circumstances, little is known of the changes that occur to the internal organisation of brain cells. It is also significant that certain membrane components of brain cells are continuously turning over and some of these are responsible for imparting to the membrane and cells their specialised functions². Thus plastic changes may sometimes result from a local alteration in the number or nature of molecules available for incorporation into the membrane, and it then becomes relevant to consider those systems which regulate the differential distribution of materials needed for maintenance and growth of cellular processes and synaptic junctions. Consequently, both here and in previous papers^{3,4} we consider the possibility that the turnover of microtubular protein (tubulin) may be involved in some of the plastic changes in the brain that result from learning or early experience. We report here significant changes in tubulin synthesis that are correlated with eye opening and the duration of a critical period.

Norwegian black hooded rats were reared under normal fluorescent lighting with a 12-h light/dark cycle (background illumination was approximately 100 lm m⁻²). At

various times after birth the rats were killed and a plug of tissue removed from area 17 of the visual cortex in each hemisphere. Area 17 was located using the maps of Adams and Forrester⁵ and Kreig⁶. Plugs of diameter 2.0 mm and weight 6.0 ± 1.5 mg were removed from each hemisphere in animals up to 1 week old. Plugs of diameter 2.5 mm and weight 10.0 ± 2.0 mg were removed from animals aged 1 to 2 weeks, and plugs of diameter 3.5 mm and weight 15.0 ± 2.5 mg were removed from animals more than 2 weeks old. Care was taken to remove the underlying white matter.

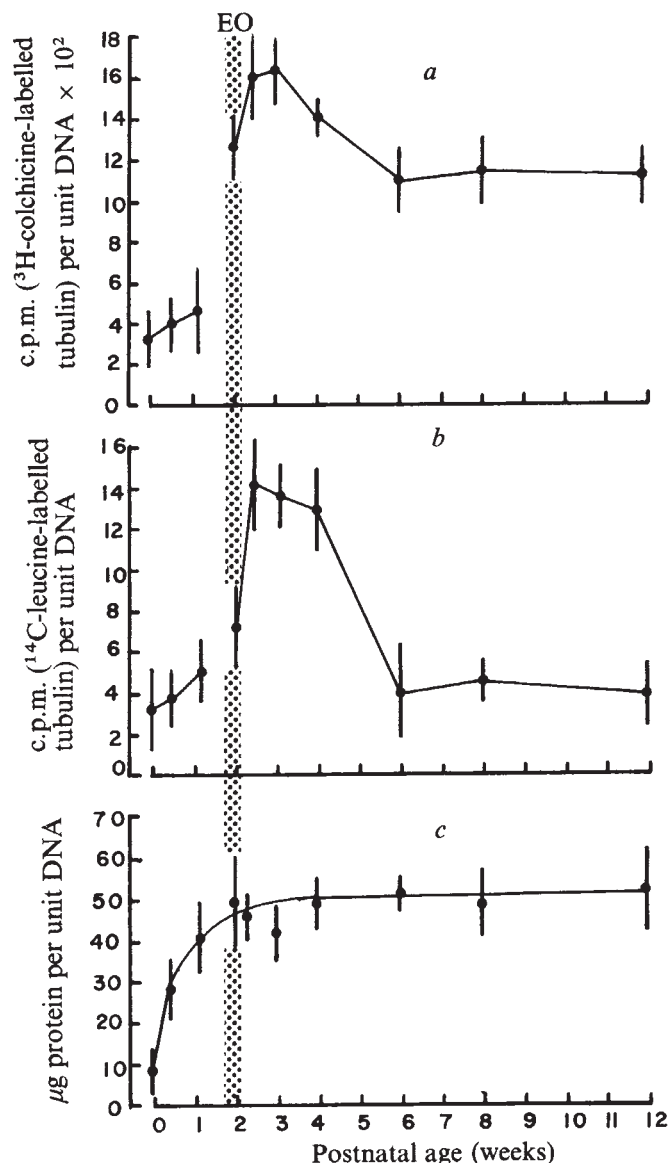


Fig. 2 *a*, Concentration of soluble tubulin in visual cortex. Ordinate shows c.p.m. ³H-colchicine (Radiochemical Centre, Amersham) bound to tubulin that has been precipitated with vinblastine sulphate (Ely Lilly & Co. Ltd). EO, Eye opening. *b*, Rate of soluble tubulin synthesis in visual cortex. Ordinate shows counts per minute ¹⁴C-labelled L-leucine (Radiochemical Centre, Amersham) in tubulin that has been precipitated with vinblastine sulphate. *c*, Protein concentration (determined by Folin-Lowry method) per (DNA per mg wet weight visual cortex). Each point represents the mean value of at least four determinations. Bars indicate standard deviations.

Both the concentration of soluble tubulin and its rate of synthesis were determined by a double labelling technique adapted from a method described by Feit *et al.*⁷. Tritiated colchicine (200 mCi mmol⁻¹) was used to assay tubulin and ¹⁴C-labelled L-leucine (348 mCi mmol⁻¹) injected into the brain ventricle 2 h before death was used to study rate of tubulin synthesis at different times postnatally. Because cortical cells increase their volume significantly in early

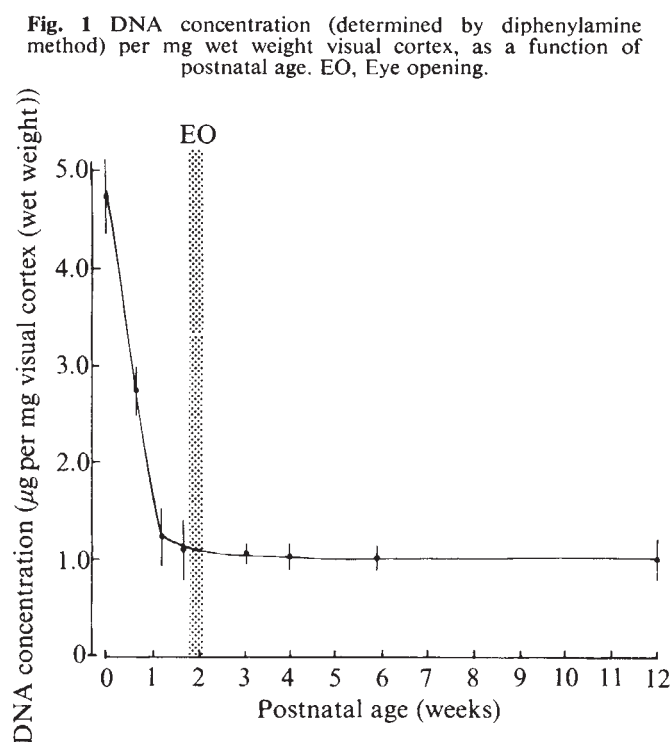


Fig. 1 DNA concentration (determined by diphenylamine method) per mg wet weight visual cortex, as a function of postnatal age. EO, Eye opening.

postnatal life we normalised our results with respect to the amount of DNA per unit wet weight of visual cortex. Although cells may change their volume, the amount of DNA remains constant per cell; consequently, the amount of DNA per unit wet weight of cortex is proportional to the number of cells (per unit wet weight of cortical tissue).

Figure 1 shows how DNA per unit wet weight of visual cortex varies as a function of postnatal age. The value declines rapidly from birth until a few days before eye-opening when the curve reaches a level which then remains constant. The decrease in DNA per unit wet weight visual cortex in early postnatal life is probably due to the fact that cells are increasing their volume and cell processes are proliferating, so that there are fewer cells per unit volume of cortex.

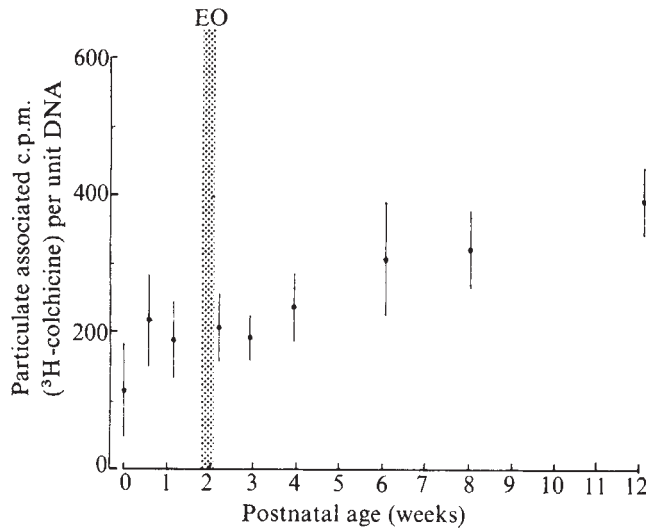


Fig. 3 Ordinate shows c.p.m. ^3H -colchicine bound to tubulin in particulate fraction.

Figure 2a shows how the concentration of soluble tubulin in the visual cortex varies with postnatal age. There is a marked rise in tubulin concentration at eye opening, and then a subsequent decline to a lower level which is maintained. This graph, however, not only represents tubulin that has been synthesised locally, but also includes tubulin that may have been synthesised elsewhere in the brain and 'imported' by cell processes and nerve fibres growing into the visual cortex from other brain regions. A more accurate picture of the changes in soluble tubulin synthesis that occur locally is given by Fig. 2b. This shows how the rate of synthesis of soluble tubulin varies with postnatal age. More especially, it indicates the amount of soluble tubulin that has been synthesised locally within the period of 2 h. As this is a relatively short period of time, it is unlikely that a great deal of newly synthesised tubulin will have been imported into our samples of visual cortex from elsewhere in the brain.

Following eye opening, both curves (Fig. 2a and b), representing tubulin concentration and rate, respectively, rise dramatically. If we consider the rate of synthesis, we see that the curve drops again by about the sixth week to approximately the level it had attained at birth, and furthermore, the rate remains constant and maintains this level thereafter into adult life.

Figure 2c shows, for comparison, how the concentration of total soluble protein in the visual cortex varies with postnatal age. The curve is quite different from that for soluble tubulin, although clearly tubulin contributes to this curve. Our results so far refer to soluble tubulin. Figure 3 shows the concentration of membrane-bound tubulin, that is, that found in the particulate fraction after high speed

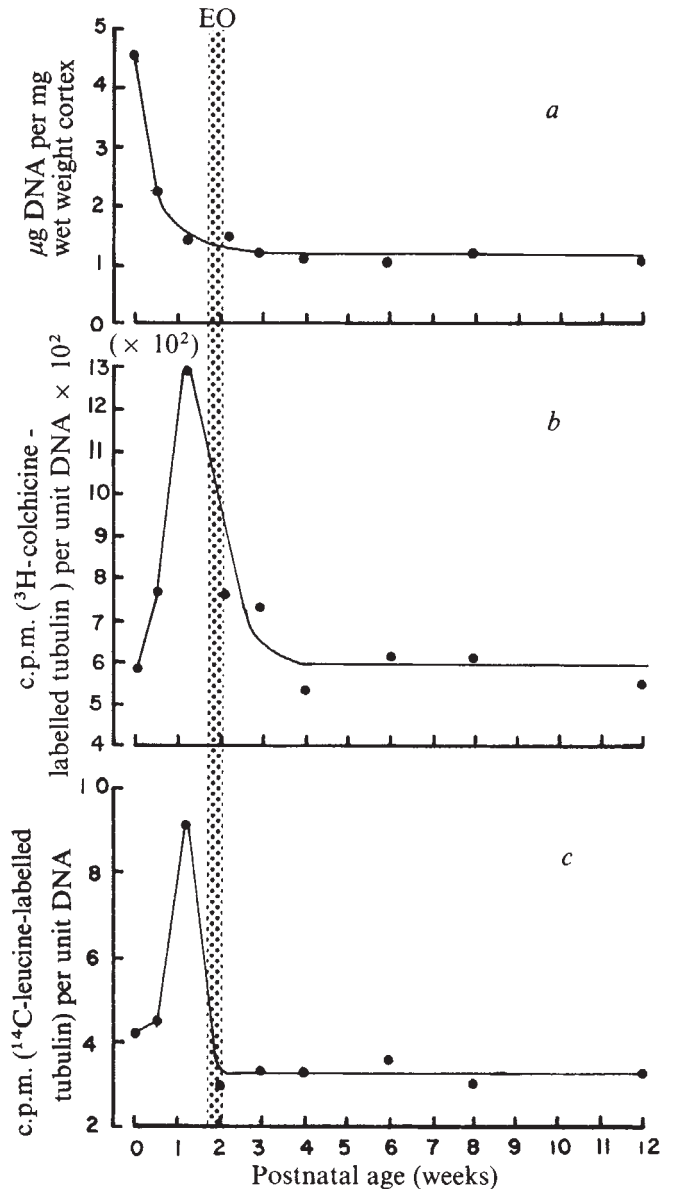


Fig. 4 a, DNA per mg wet weight cerebral cortex. b, Concentration soluble tubulin cerebral cortex. c, Rate of synthesis soluble tubulin cerebral cortex.

centrifugation, and again we find no obvious similarity to our previous curves for soluble tubulin.

An important control is whether the correlation between the change in tubulin synthesis and eye opening is region-specific and restricted to the visual cortex, or whether it is also a characteristic of the whole brain. Figure 4 shows the corresponding measurements of concentration and rate of synthesis of soluble tubulin for the whole cerebral cortex. Again it is clear that the curves show no obvious correlation with eye opening and are similar to results obtained by previous workers for whole brain⁸.

What functional significance may we attribute to this pattern of tubulin synthesis in the visual cortex? First, although the behavioural and electrophysiological evidence for the existence of a 'critical period' in rat visual cortex is meagre, we wish nevertheless to suggest that the peak in tubulin synthesis, which extends from eye opening until about the sixth week after birth may be correlated with a transient 'critical period' of increased plasticity and susceptibility to visual deprivation. Indeed, our 'biochemical estimate' of what we believe to be a 'critical period' in rat visual cortex, agrees broadly with the behavioural data of Walk and Walters⁹ who have carried out experiments using

the 'visual cliff' to examine the effects of dark rearing on the development of depth perception in the rat. It also agrees with the more recent behavioural data of Tees¹⁰.

From a physiological standpoint, the consequence, to the cell, of having a sudden and large increase in the size of its available pool of soluble tubulin may be to enable the cell to maintain temporarily an uncommonly large number of synapses and cytoplasmic processes during the critical period. When the level of tubulin drops there may be a corresponding reduction in the number of microtubules since their subunit 'tubulin' only has a half life of about 4 d. Likewise, many cellular processes and connections may regress because there are insufficient microtubules to transport and keep them supplied with the essential materials needed for their maintenance.

At present, we are not yet able to affirm a causal relationship between eye opening and the sudden rise in tubulin synthesis. The answer here must await the results of experiments with dark-reared rats which are now in progress. Also, the possibility that genes controlling tubulin synthesis may be switched on by a sudden increase in neuroelectric activity, brought about by eye opening, is another hypothesis for which we hope soon to have an answer. Indeed, several examples are already known where neuroelectric activity at synaptic junctions affect gene action^{11,12}; for example, in the autonomic nervous system, Thoenen¹² has shown that adrenal tyrosine hydroxylase activity may be increased by nervous activity, and his experiments provide evidence that the triggering of an operon regulating synthesis of noradrenaline may be brought about by neuroelectric activity. Clearly then, if during the 'critical period' in the visual cortex, major changes in macromolecular, and in particular, 'tubulin' synthesis are triggered by neuroelectric activity, this could in part account for the alteration in neuronal morphology and functional organisation that results from restriction of visual experience in early life. Answers to these questions must, however, await further experiments.

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In vivo evidence for a circadian rhythm in membranes of *Gonyaulax*

It has been suggested that rhythmic oscillations in membranes are the underlying mechanism for endogenous circadian rhythms^{1,2}. To support this suggestion, periodic oscillations in membranes of organisms displaying circadian rhythmicity must first be demonstrated. Rhythmic oscillations associated with membranes have been observed in the

spontaneous firing of the optic nerve of *Aplysia*³ and in the transmembrane potential of the pulvini of *Samanea*⁴. It is not clear if these membrane-associated rhythms are a function of specialised cells, or are basic phenomena of circadian rhythms. The only membrane-associated rhythm described in a unicellular organism so far is that of a circadian rhythm in particle distribution of one of the membranes of *Gonyaulax polyedra*⁵. We have now investigated the rhythmic changes in the membrane potential of *G. polyedra*. We used the method of Hoffman and Laris⁶, in which fluorescent cyanine dyes are used to monitor the membrane potential *in vivo* of cells not amenable to the insertion of microelectrodes, in this case because of their small size and lack of a central vacuole. Our results suggest a temporal reorganisation of one of the membranes of *Gonyaulax* with circadian time.

G. polyedra 70A, a clonal strain isolated in 1970 by one of us (B.M.S.), was maintained in modified f medium (f/2) (ref.7), on an alternating 12-h light-dark schedule (1,500 $\mu\text{W cm}^{-2}$, 21 °C). Cultures were transferred to continuous light (300 $\mu\text{W cm}^{-2}$, 21 °C) at the beginning of the light period (0 circadian time, CT). At approximately 6-h intervals thereafter, samples of cells were collected by gentle centrifugation, washed three times with 10 volumes of 0.5 M NaCl, 1 mM Tris(hydroxymethylaminomethane)-HCl, pH 8.1, and finally suspended in 1 ml of the same solution. Next, 0.2 ml of the suspension containing 2.5×10^4 cells was added to 2 ml of 100 mM NaCl, pH 8.1 (K^+ -free medium). After equilibration for 15 min with stirring at room temperature (22-23 °C), 10 μl of a stock solution (0.5 mg per ml ethanol) of the fluorescent cyanine dye, 3,3'-dipropylthiodicarbocyanine iodide (designated diS-C₂(5)), supplied by Dr Alan Waggoner of Amherst College, was added. After fluorescence decay had stabilised (approximately 2 min), 10 μl of stock solution of valinomycin was added to promote potassium transport⁸. Finally, increasing amounts of 4 M KCl were introduced into the suspension (Fig. 1). The methods used to record fluorescence have been described before⁹. The data at each circadian time were plotted as the change from the steady state fluorescence (ΔF) in the presence of valinomycin as a function of the

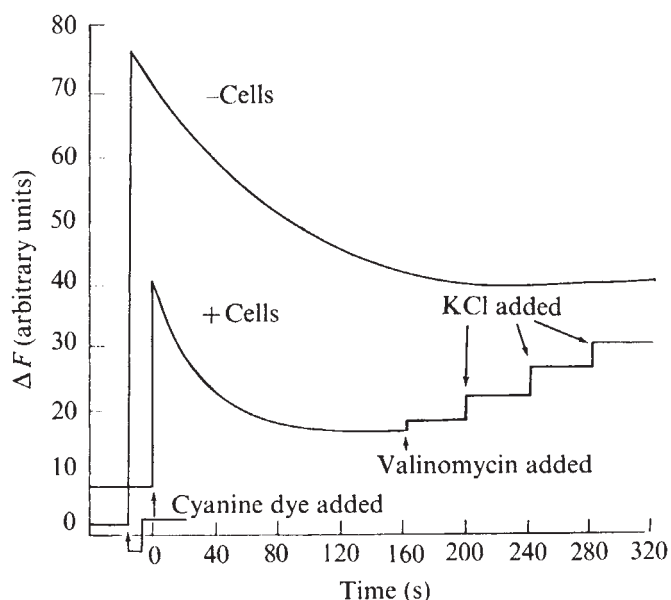


Fig. 1 Schematic of a typical trace of changes in fluorescence intensity of the dye diS-C₂(5) as a function of time in 100 mM NaCl-Tris medium, pH 8.1, in the presence (+) and absence (-) of 2.5×10^4 cells ml^{-1} . Excitation was at 622 nm and emission was recorded at 670 nm. Final concentrations of chemicals added as indicated were: dye, 2.4×10^{-6} M; valinomycin, 6×10^{-5} M, ethanol, 1% v/v, and 64 mM KCl (added as 4-M KCl in 5-, 10-, 50-, and 100- μl increments).

logarithm of the external concentration of potassium $[K^+]_o$.

Gonyaulax, a marine phytoplankter, is normally grown in a medium of approximately 500 mM salt. But at concentrations greater than 300 mM salt, dye fluorescence substantially diminishes. Therefore, a 100 mM NaCl (containing no potassium) solution was chosen for the determinations. In this solution cells were observed not to lyse, or lose potassium (as determined by flame photometry), and maintained their motility.

Gonyaulax has a complex cell covering consisting of several membranes external to the spheroplast^{9,10} and it was necessary to determine which membrane the fluorescent cyanine dye probes. When *G. polyedra* cells are centrifuged and stirred, membranes exterior to the spheroplast¹⁰ lose their permeability characteristics. This is readily observable using the mortal stain Evans blue dye¹¹ and the vital stain neutral red¹². It seems that the only likely membrane over which a membrane potential could be maintained is the spheroplast-limiting membrane. We observed no change in the steady-state level of dye fluorescent intensity on addition of potassium in the presence of valinomycin to *G.*

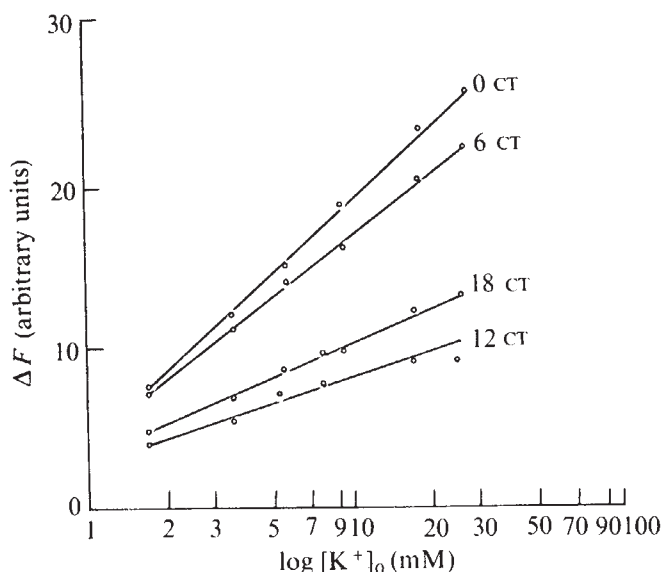


Fig. 2 Change in the steady-state level of fluorescence intensity (ΔF) against $\log[K^+]_o$, conditions as in Fig. 1, at different circadian times. Cells were maintained for approximately 3 d under constant illumination and temperature before and during sampling.

polyedra cells mechanically ruptured, to isolated cell-wall fractions¹⁰, and to cells maintained for 25 min at 0–10 °C and 30 °C. Since the cyanine dyes monitor changes in membrane potential of cells or subcellular organelles only if their limiting membrane is intact⁶, we conclude that the dye monitors changes associated with the spheroplast membrane of *Gonyaulax*. Furthermore, in *Amphiuma* red blood cells and Erlich ascites tumour cells, which have internal membranes and organelles, the dye probes the plasma membrane and not the internal membranes^{6,13}.

The interaction of the cyanine dyes with membranes and the mechanism by which these dyes monitor the membrane potential are not completely understood^{14,15}. But a decrease in fluorescence intensity and a redshift (3–4 nm) in the emission spectrum of the dye is characteristic of the interaction of the dye and biological and model membranes^{6,14}. The emission spectrum of the dye diS-C₃(5) in the presence of centrifuged and stirred *G. polyedra* results in a characteristic decrease in the intensity of fluorescence of the dye, and a 4-nm redshift in the emission spectrum.

In previous studies with various preparations, the fluorescence of the cyanine dye decreased in intensity as the potential became more negative (internally), and increased

as it became more positive⁸. The decrease was apparently due to binding, and the increase to release of the dye from the membrane¹⁴. We were not able to observe a decrease in fluorescence intensity indicative of hyperpolarisation on addition of valinomycin to cells in potassium-free medium, although flame photometry showed that addition of valinomycin to cells in K^+ -free medium resulted in increased membrane permeability to potassium. The most likely explanation for the lack of a decrease in the intensity of fluorescence with valinomycin in K^+ -free medium is that the internal potential was already very negative and that the further hyperpolarisation was not large enough to be detected by the dye. This situation is observed with mitochondria in the energised state⁸. No independent method is available to resolve this possibility. Since a decrease in fluorescence intensity could not be observed, the membrane potential could not be measured by the "null point" method⁸, thus whether or not the absolute value of the membrane potential changes with circadian time could not be determined. But the addition of potassium to cells in the presence of valinomycin led to an increase in fluorescence intensity indicative of depolarisation⁸. Plots of ΔF against $\log [K^+]_o$ in the presence of valinomycin were linear over most of the range studied (Fig. 2), and the shape of these curves is typical of curves seen with other preparations⁸. The relationship suggests that in the presence of valinomycin the potential is shifted towards the K^+ equilibrium potential⁸. Therefore, plots of ΔF against $\log [K^+]_o$ in the presence of valinomycin may be indicative of the relative influence of potassium plus valinomycin on the existing potential difference across the membrane^{6,8}.

Although it is conceivable that oscillations in the concentration and fluorescence intensity of certain pigments could interfere with dye fluorescence emission, no oscillations in the total concentration and fluorescence intensity of chlorophyll *a* and *c* have been observed with circadian time (unpublished results of M.A., B. Prézelin and B.M.S.).

The initial events of cyanine dye-cell-valinomycin interactions (dye fluorescence equilibration time and slight increase in fluorescence with valinomycin (Fig. 1)) appeared constant over circadian time and suggested that the initial attachment of dye and valinomycin was not changing significantly. Plots of ΔF against $\log [K^+]_o$ determined at various times in the circadian cycle generated curves with slopes (linear portion) which varied between two extremes (Fig. 2). A plot of the slope against circadian time showed that the

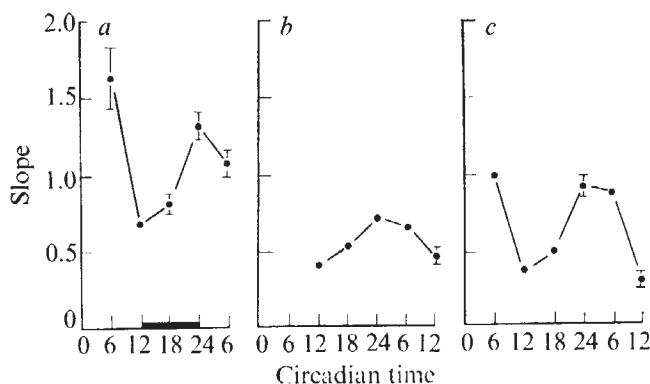


Fig. 3 Plot of the slope, calculated from changes in fluorescence intensity against $\log[K^+]_o$ at various times in the circadian cycle (Fig. 2), as a function of circadian time from three independent experiments. *a*, Cells were maintained on a 12-h light-dark schedule; black bar represents dark period. *b* and *c*, Cells maintained at constant illumination and temperature, 1 and 3 d respectively, before and during sampling. The bar associated with some points represents the extreme values measured. Where no bar is shown, the values fall within the circle.

slope varied rhythmically with a 24-h periodicity (Fig. 3). A steep rise in the slope occurred before 0 CT and 24 CT (both corresponding to dawn) followed by a rapid decline observed at 12 CT (corresponding to dusk). The rhythmic oscillation in slope was observed both in a light-dark cycle and after 4–5 d in constant conditions (Fig. 3), characteristic of an endogenous circadian rhythm.

The cyanine dye, diS-C₂(5), seems to monitor some *in vivo* rhythmic phenomenon(a) of the spheroplast membrane, but it is not clear how the changes in slope are to be interpreted. Changes in fluorescence on addition of extracellular potassium and hence changes in slope with circadian time, were observed only in the presence of cells, cyanine dye and valinomycin together, and at approximately room temperature (22–23 °C). When such determinations were made with cells maintained below 10 °C or above 30 °C for 25 min, temperatures at which *Gonyaulax* cells apparently become leaky (unpublished observations of M.A. and B.M.S.), plots of ΔF against $\log [K^+]_0$ generated slopes with values near zero. Although this might suggest that, at physiological temperatures (16–24 °C), changes in slope may indicate a circadian rhythm in membrane permeability to ions other than K⁺ in the presence of valinomycin, no rhythmic changes in [Na⁺], [Cl[−]], [Mg²⁺], and [Ca²⁺] were detected in *Gonyaulax* by flame photometry (ref. 16 and unpublished data on *Gonyaulax* ion assays of L. Anderson and B.M.S.). Furthermore, no change was detected in the steady state of dye fluorescence on addition of increasing amounts of 4 M NaCl to a suspension of cells in the presence or absence of valinomycin. In addition, rhythmic oscillations in total protein, determined by the Lowry¹⁷ and fluorescamine methods¹⁸, were not observed. Together these observations suggest that neither the permeability of the membrane to these ions, nor possible rhythmic oscillations in the concentration of non-diffusible anions, can account for the observed rhythmic oscillations in slope.

The constant field equation predicts that the slope of plots of ΔV_m (membrane voltage, ΔF in this case) versus $\log [K^+]_0$ will increase with increasing permeability of potassium relative to other ions⁶. In this study valinomycin was used to increase the permeability of potassium specifically. Therefore, it seems that the dye monitors rhythmic changes in the relative influence of potassium plus valinomycin on the existing spheroplast membrane potential, and that these changes reflect rhythmic oscillations in the permeability of the membrane to potassium mediated by valinomycin. The kinetics of valinomycin-mediated K⁺ transport are significantly influenced by the dynamic state and chemical composition of the membrane^{19–23} and the use of valinomycin-mediated potassium transport has been suggested as a potential probe for membrane structure²⁴. It is conceivable that the circadian rhythm in valinomycin-mediated transport of potassium and its subsequent effect on the degree to which the membrane can be depolarised implicates a rhythmic alteration in the physicochemical nature of the spheroplast membrane. Whether this includes a rhythmic change in the absolute value of the membrane potential before the addition of potassium and valinomycin could not be determined in this study. The existing membrane potential, however, may be differentially influenced by the presence of potassium and valinomycin at particular times in the circadian cycle. The existence of rhythmic alterations of the spheroplast membrane is further supported by evidence for a circadian rhythm in the fragility of the spheroplast membrane (unpublished results of M.A., B. Prezelin and B.M.S.) and in the composition of the polyunsaturated fatty acids of *Gonyaulax*²⁵. Both these circadian rhythms occur at approximately the same phase (0 CT) in the circadian cycle as the rhythmic oscillation in slope observed in this study. Whether these rhythms constitute part of the underlying oscillator or are only driven by it remains to be investigated.

In conclusion, the evidence presented here which suggests an *in vivo* circadian rhythm in the relative influence of potassium and valinomycin on the existing electrical properties of the spheroplast membrane implies a rhythmic temporal reorganisation of the membrane, and supports the suggestion that membrane oscillations are involved in biological clock phenomena. Apart from its significance in the mechanism of the circadian oscillator, a circadian rhythm in membrane temporal reorganisation could account for the large discrepancies in permeability data between laboratories²⁶, and should be considered in future studies of biological membranes.

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Genetics of chloroquine resistance in malaria parasites

THE resistance of the malaria parasite of man, *Plasmodium falciparum*, to treatment with chloroquine is a growing problem, especially in South-east Asia and South America¹. It is not known whether the emergence of resistance is attributable to the selection of resistant mutants under drug pressure, to the spread of naturally resistant forms in the parasite population or to adaptation to the drug by previously sensitive parasites. In malaria parasites of rodents, it has been shown that resistance to the antifolate drug pyrimethamine arises by mutation² and that the genetic factors involved can undergo recombination with other markers in crosses between resistant and sensitive parasite lines³. Resistance to chloroquine in rodent plasmodia seems to take several forms. *P. yoelii* isolates are innately resistant to the drug⁴, whereas *P. berghei* and *P. vinckei* are sensitive. Stable chloroquine resistance has been

produced in *P. vinckei* by drug selection in the laboratory⁵ but resistance developed in this way in *P. berghei* is usually unstable in the absence of the drug⁶.

I have investigated the genetic basis of chloroquine resistance in another rodent malaria species, *P. chabaudi*, which I believe serves as a useful model for *P. falciparum*. A chloroquine-resistant parasite line (411 AS) of *P. chabaudi* was obtained in mice by submitting blood forms of a sensitive line to a continuous low level drug pressure. Five mice (C57BL, 4–5 weeks old) were each injected with 10⁶ blood forms and treated with oral doses of chloroquine at 2 mg kg⁻¹ daily for five days from the day of inoculation. Mice were weighed individually at the start of treatment and on day four, the drug dose being adjusted if any change in weight had occurred. Parasites from that mouse which exhibited the highest parasitaemia were injected into a second group of mice and the treatment repeated. Five similar passages were made in mice, the dose being increased to 3 mg kg⁻¹ from the third passage. Parasites which survived this course of treatment were then transmitted through mosquitoes into mice and tested for resistance. The line survived treatment of 3 mg kg⁻¹ given for six days, whereas the drug-sensitive parent line, which had undergone a similar series of passages without drug pressure, was eradicated.

Table 1 Characterisation of 70 clones, produced by mixed infection of mosquitoes by lines 411AS and 96AJ

LDH form*	6PGD form†	Pyrimethamine response‡	Chloroquine response	Number of clones
2	3	S	S	32
2	3	S	R	11
2	3	R	S	0
2	3	R	R	0
2	2	S	S	2
2	2	S	R	7
2	2	R	S	0
2	2	R	R	0
3	3	S	S	1
3	3	S	R	2
3	3	R	S	2
3	3	R	R	1
3	2	S	S	1
3	2	S	R	7
3	2	R	S	0
3	2	R	R	4
Total				70

*LDH2 or 3, Electrophoretic forms of the enzyme lactate dehydrogenase.

†6PGD2 or 3, Electrophoretic forms of the enzyme 6-phosphogluconate dehydrogenase.

‡R, resistant to drug pressure; S, sensitive to drug pressure.

The stability of the resistance was tested by keeping the parasite line undrugged for 25 blood passages (6 months), tests for resistance being carried out at passages 10, 15 and 25. At passage 15, parasites were also transmitted through mosquitoes into mice and tested for resistance. In all tests (3 mg kg⁻¹ for 6 d) the parasites were found to be resistant, the resistance thus proving stable in the absence of drug pressure.

The genetic basis of the chloroquine resistance was investigated in a cross between line 411AS and a sensitive line denoted 96AJ. The two lines differed additionally in three characters. Line 411AS was resistant to pyrimethamine (15 mg kg⁻¹ administered for four consecutive days after inoculation of parasites), and possessed electrophoretic forms of the enzymes 6-phosphogluconate dehydrogenase (6PGD-2) and lactate dehydrogenase (LDH-3) respectively⁷. Line 96AJ was sensitive to both drugs and possessed enzyme forms 6PGD-3 and LDH-2.

The cross was performed using a technique described previously³. Equal volumes of parasitised red blood cells of each line were mixed and injected intravenously into a splenectomised rat, in order to obtain increased numbers of gametocytes. Five days later, mosquitoes (*Anopheles stephensi*) were permitted to feed on the mixed infection. At this stage micro- and macrogametes from each line were formed and, as a result, both cross

and self fertilisation occurred. Fourteen days later, the infected mosquitoes were permitted to feed on mice. After mosquito transmission, the blood forms which developed were cloned by dilution, and each clone examined for drug response and enzyme type. As controls, the parent lines 411AS and 96AJ were transmitted through mosquitoes separately, and tested for drug response and enzyme type in a similar way. In tests for chloroquine resistance, 10⁶ parasites of each clone were injected into two mice, which were treated with six daily doses of chloroquine at 3 mg kg⁻¹. Pyrimethamine resistance was tested similarly using doses of 15 mg kg⁻¹ for 4 d. Electrophoretic forms of 6PGD and LDH were detected as in the method of Carter⁷. Seventy clones were derived from the products of the cross, the characteristics of which are shown in Table 1. Clones showing recombinant as well as parental type characters were present. The numbers of clones of the two parental types were significantly different, there being 32 of type 96AJ but only 4 of type 411AS. This suggests that selection against line 411AS had occurred, either when the parental lines were growing together in the rat before mosquito transmission or between zygote formation and the time at which clones were established. No significance, therefore, can be attached to the number of clones found in each recombinant class. It is of interest, however, that the proportion of chloroquine resistant to chloroquine sensitive clones found is 32:38, suggesting that the resistant form has no disadvantage compared to the sensitive form.

The results show that the chloroquine resistance which developed in *P. chabaudi* is a stable character, is inherited in simple Mendelian fashion, undergoes genetic recombination with other markers, and probably arose by mutation and selection in presence of the drug. Although the level of resistance developed is low, the treatment of 3 mg kg⁻¹ for six days is sufficient to eliminate sensitive forms, and is similar to the recommended doses for suppression of human malaria⁸.

The results, therefore, may provide an explanation of how chloroquine resistant forms could arise in the human malaria parasite, *Plasmodium falciparum*, in areas where the drug is widely used.

Further work is in progress in this laboratory to determine how the genetic factors involved can spread in populations of sensitive parasites. This knowledge is of particular importance as chloroquine resistance in *P. falciparum* is widespread in regions such as South-east Asia, but not yet in Africa.

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Doubling potential of fibroblasts from different species after ionising radiation

It is well known that chicken fibroblasts invariably die after a certain number of doublings *in vitro* (cell senescence) and that they have never been established by any chemical or physical agent¹. On the other hand, mouse fibroblasts invariably acquire spontaneously an infinite growth potential, and transformation can be induced at a high frequency

by different oncogenes². Other species like man also yield fibroblasts which never acquire spontaneously the capacity to divide for ever³ but can become permanent cell lines after infection with SV40 virus⁴. Transformation in human fibroblasts has only been unequivocally demonstrated after treatment with certain viruses. This behaviour of fibroblasts *in vitro* has been attributed to different nutritional requirements⁵. The two approaches to explain the phenomenon can be summarised as intrinsic properties against environmental conditions. We have previously shortened the life-spans of cultivated chicken fibroblasts with low dose rate ionising radiation⁶. We have carried out the same type of experiment with human and mouse fibroblasts and found that the response to ionising radiation fits the relative tendencies of these different fibroblasts to yield permanent cell lines.

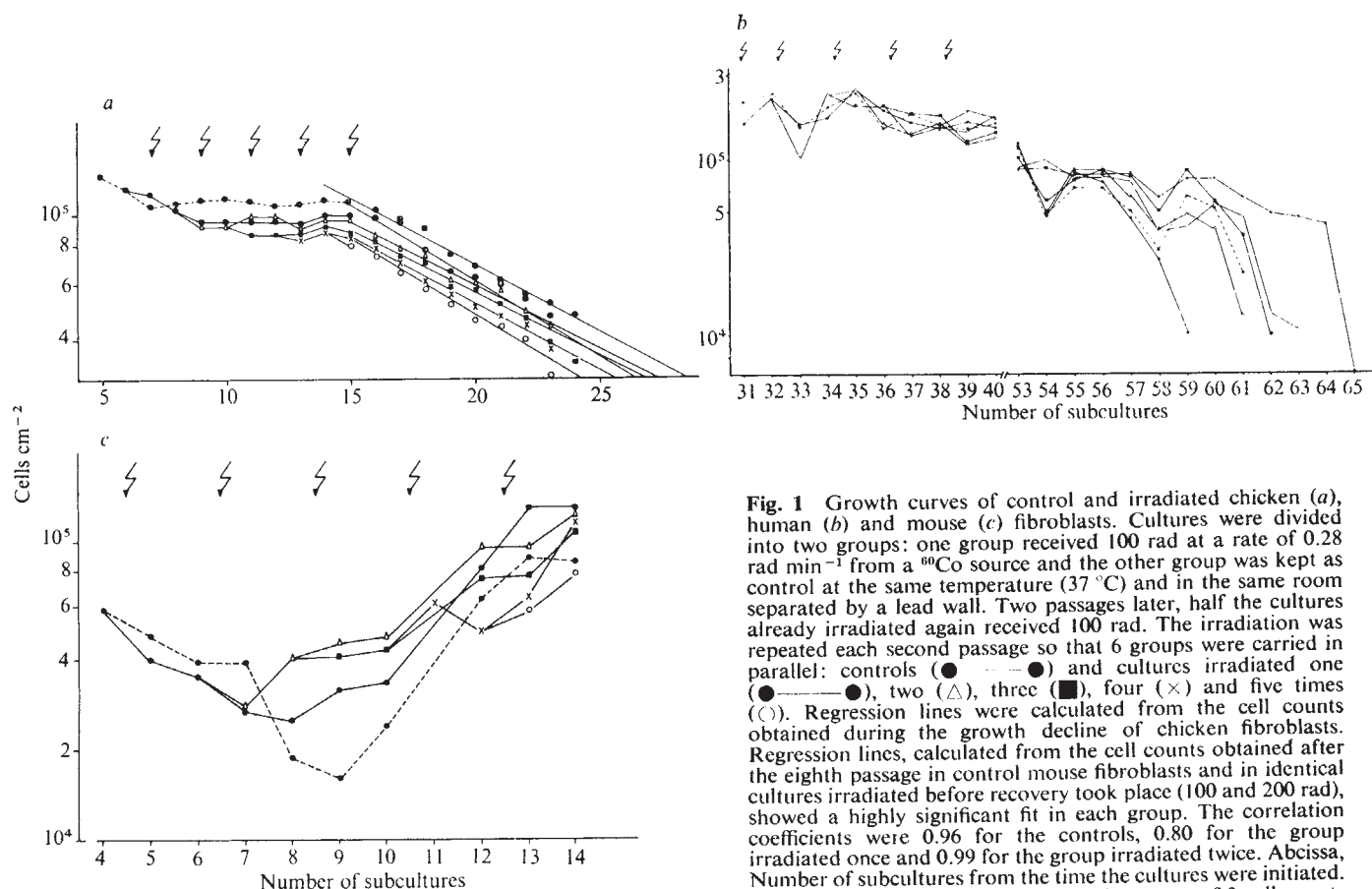
Human embryonic lung fibroblast and mouse newborn lung fibroblast cultures were started as described previously³. The same nutrient medium (Eagle's MEM with 10% foetal calf serum) was used to cultivate these two types of cell. The initiation and maintenance of chicken embryonic cultures have also been reported previously⁶. Tryptose phosphate broth (5%) was added to the Eagle's medium used to cultivate the chicken fibroblasts, to improve the growth of these cells. The irradiation was started during the phase of active proliferation. The growth curves (Fig. 1) of irradiated chicken fibroblasts were parallel to the growth curve of the controls, but the former had a lower saturation density and stopped dividing earlier. This decrease in the saturation density and lifespan was directly proportional to the number of radiation dose received.

In human fibroblasts the saturation densities of the irradiated cultures oscillated around the saturation density of the controls. Cultures irradiated with 100 rad, however, stopped dividing earlier than the controls. The cultures irradiated with 200, 300 and 500 rad had the same lifespan as control cultures, and the cultures irradiated with 400 rad had the longest survival.

With mouse cells the establishment of the control cultures followed the pattern described previously⁷. First, there was a progressive decline in the maximal number of cells (time when the number of mitoses was negligible); around passage 9 the cells recovered from the growth decline, the maximal cell density increased progressively and finally the population became established with a saturation density that stabilised around 10^5 cells cm^{-2} from passage 20 onward. The growth curves of the irradiated cells were of the same type as in the controls but the recovery occurred earlier.

Calculation of the total amount of cells produced in controls and in irradiated cultures gave different results in each species (Fig. 2). With chicken fibroblasts the total amount of cells produced in each group decreased logarithmically with increasing doses of radiation received. With human fibroblasts the total number of cells was identical in all groups so that there was no slope. Finally, with mouse fibroblasts the total amount of cells produced increased logarithmically and was directly related with the radiation dose received.

These results indicate that ionising radiation accelerates a natural phenomenon: in cells with a limited growth potential (chicken) it shortens the lifespan and in cells that can acquire an unlimited growth potential (mouse) it



before subcultivation. These curves represent the number of live cells present in the cultures before each subcultivation. Since at each passage the inoculum is always half of the number of cells found at resting stage (1:2 split), the curves merely express if the population doubled or not after each subcultivation. Number of non-dividers not known.

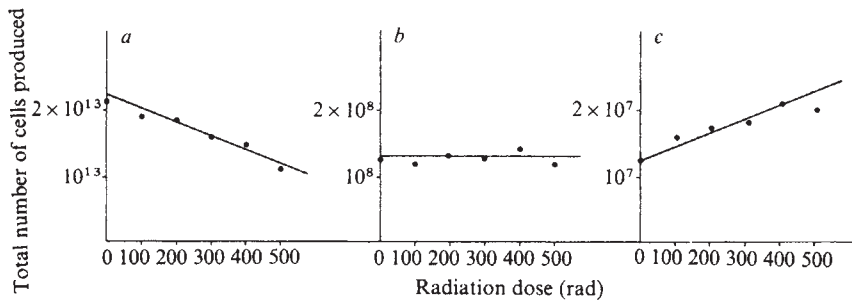


Fig. 2 Total amount of cells produced in control and irradiated cultures in the experiments illustrated in Fig. 1. In mouse fibroblasts the total number of cells produced was calculated up to passage 20—that is, at the time when the saturation densities became stabilised and identical in all groups. *a*, Chicken; *b*, human; *c*, mouse.

accelerates the acquisition of the latter; human fibroblasts showed an intermediate response since ionising radiation neither established the cultures as with mouse cells nor reduced the number of cells produced, as with chicken fibroblasts. Human cells also show an intermediate behaviour to chicken and mouse fibroblasts in the sense that chicken cells never establish either spontaneously or after treatment with virus, chemicals or radiations, mouse cells establish spontaneously, and human cells never establish spontaneously but can establish after infection with certain viruses. So they seem more prone to become established than chicken cells, but less than mouse cells.

It is difficult to see what could cause the different responses of the fibroblasts from these three species. It seems, however, that there are three possible explanations. It is possible that in the case of mouse fibroblasts there are some cells in the original population which have the potential of dividing indefinitely. If these cells are less radio-sensitive, ionising radiation could favour their overgrowth by killing or retarding the growth of the other cells in the population which have a limited doubling potential. In that case, one would have to infer that in chicken fibroblast populations there are not cells with an infinite doubling potential. On the other hand, among human fibroblasts there would be clones with different growth potentials, and ionising radiation would favour the clones with the longest division potential.

The other hypotheses would concern the presence or absence of repair mechanisms or of a repairable substrate. It has been suggested that malignancy could be due in certain cases to mutations caused by faulty repair and that situations inducing repair therefore increase malignant transformation⁸. Repair induced by low dose rate ionising radiation would cause mutations which determine the capacity to divide indefinitely. The situation would be analogous to what happens with *Micrococcus radiodurans* that becomes resistant to ionising radiation, because it develops the capacity to repair quickly the DNA lesions⁹. If this were the case one would have to postulate that cells like mouse fibroblasts are endowed with the capacity to spontaneously undergo these changes, which would be accelerated by ionising radiation. Cells like chicken fibroblasts, on the other hand, are endowed with a finite lifespan, perhaps because they have no repair enzymes or because the substrate (for example, DNA) has a molecular organisation which would impede the action of these enzymes. In any case the results seem to provide further evidence that a finite or infinite lifespan is an intrinsic property of cells and is not just dependent on a particular environment.

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Absence of oxygen effect for induction of resistance to ionising radiation

SURVIVAL curves for *Chlamydomonas reinhardtii* exposed to ionising radiation have an initial 'shoulder' region where sublethal damage is accumulated, followed by an exponential region where a further single event will result in cell death. When an initial dose is given to cells, sufficient to reduce the surviving fraction to the exponential region, followed by a series of graded doses given some hours later, cells recover from sublethal injury inflicted by the first dose, as shown by the return of the shoulder region on the second dose survival curve. The slope of the exponential region of the second dose survival curve is smaller than that of a single dose survival curve¹. Thus cells have become more resistant as a result of exposure to a

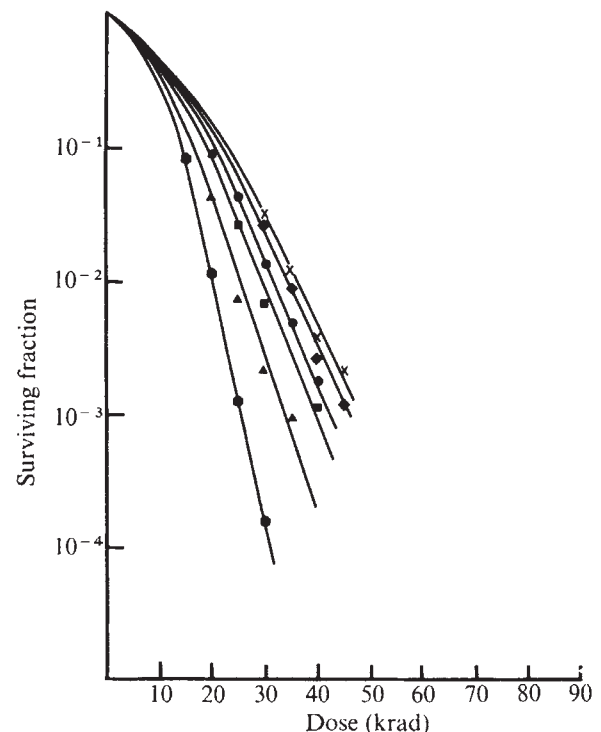


Fig. 1 Survival curves for *Chlamydomonas* given either single doses (●) or fractionated doses at different times after a first dose of 15 krad given in anoxic conditions 1 h (▲), 2 h (■), 3 h (●), 4 h and 5 h (×) between doses. Split dose curves have been normalised with respect to the surviving fraction after the first dose.

first dose. A similar effect has been reported for a line of HeLa S3 cells². The increase in resistance observed in these two types of cell has been interpreted¹ as resulting from an increase in the capacity of cells to repair radiation damage. An alternative hypothesis³ that could account for the observed change in sensitivity proposes that cells sublethally damaged by the first dose of radiation are repaired during the interval between doses, so as to render the intracellular sites less susceptible to damage from a second dose of radiation. The experiments reported here cast doubt on the validity of the latter hypothesis.

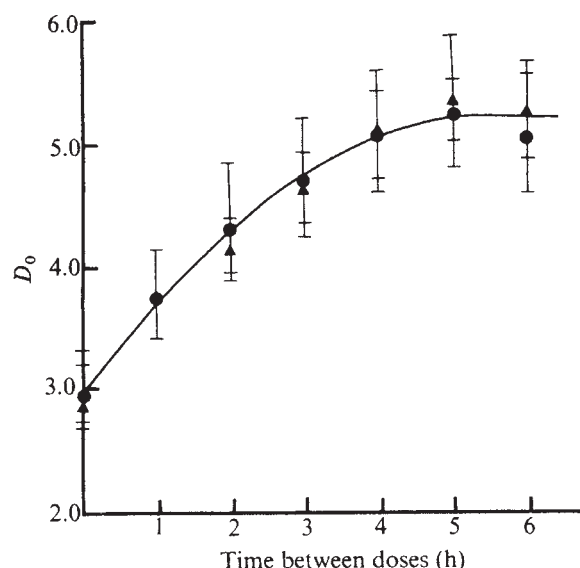


Fig. 2 D_0 as a function of time between doses given in aerobic conditions after a first dose of 15 krad given in aerobic (▲) or anoxic (●) conditions. Vertical bars represent 95% confidence intervals.

I also report that irradiation of cells in aerobic or anoxic conditions was equally effective in inducing an increase in resistance of cells to a second series of doses. That is, the oxygen enhancement ratio (OER) was 1.0 for induction of resistance, in contrast to the OER of 2.2 observed for cell killing. The OER for cells killed by a second series of doses given some hours after an initial dose was the same as that for cells killed by single doses.

Alper has postulated that radiation causes two types of cellular lesions both of which can contribute to cell death^{4,5}. One, called type N, occurs in the DNA of the cell and is subject to little or no enhancement by oxygen while the other, called type O, occurs in cell membranes and is subject to a large enhancement by oxygen. It has been postulated that after irradiation differential repair can occur in these two intracellular regions, resulting in variations in resistance of cells and in the OER for cell death. Radioresistance may thus result from repair to DNA which will result in a large OER value because killing will be due to events occurring in the cell membranes or membrane-associated structures. Radioresistance may also result from repair to membranes or membrane-associated structures which will result in a lower OER because killing will be due predominantly to events occurring in the DNA. Alper has shown for variants of *Escherichia coli* strain B that a proportionality exists between the resistance of the variants to radiation and their OER values, and has postulated that this occurs because the more resistant bacteria can repair type N damage. Wild-type forms of *C. reinhardtii*⁶ and the slime mould *Dictyostelium discoideum*⁷ have values of OER of about 2.2 and 1.8 respectively whereas their radiosensitive mutants have values of OER of 1.0 or less. The more radioresistant wild types of these organisms were also shown to be resistant to ultraviolet light of a wavelength absorbed strongly by nucleic acid, suggesting that the resistance in these cases to ionising radiation may result from the cells ability to repair damage to DNA.

In the experiments reported here the resistance of *Chlamydomonas* to a second series of doses was increased by giving an initial dose of radiation followed by an interval before the second dose. It was therefore surprising, in view of the results mentioned above, to find that the OER was unchanged for these more resistant cells.

The methods used in the culture of vegetative cells of *C. reinhardtii* have been described previously^{8,9}. Cells were irradiated in suspension in a liquid medium held in a glass vessel and bubbled with either air or nitrogen (BOC white spot grade). To achieve anoxic conditions, nitrogen was bubbled for 10 min before and during irradiation. Irradiations were performed in the dark. Samples were irradiated with 7 MeV electrons from the MRC linear accelerator at a dose of 20 krad min⁻¹. Doses were monitored with an ionisation chamber which had been calibrated against a Baldwin-Farmer secondary standard dosimeter, and checked by the ferrous sulphate method.

Table 1 Oxygen enhancement ratios for cells given single and split doses

Treatment	Oxygen enhancement ratio
Single doses	2.18 (2.00–2.37)
Second series of doses following an initial conditioning dose of 15 krad given in aerobic conditions	2.25 (2.06–2.46)
Second series of doses following an initial conditioning dose of 15 krad given in anoxic conditions	2.22 (2.08–2.36)

Figures in parentheses are 95% confidence intervals.

During intervals between doses, suspensions of cells were shaken gently in conical flasks in the light at 22 °C. After irradiation and appropriate dilution, cells were plated on membrane filters (Oxoid) resting on yeast extract agar (Oxoid). Colonies were scored after 3 d of growth under continuous illumination.

Experiments in which cells were given a first dose in either aerobic or anoxic conditions, followed by a graded series of doses in aerobic conditions at various times (Fig. 1), showed that the final slopes of the split dose survival curves decreased

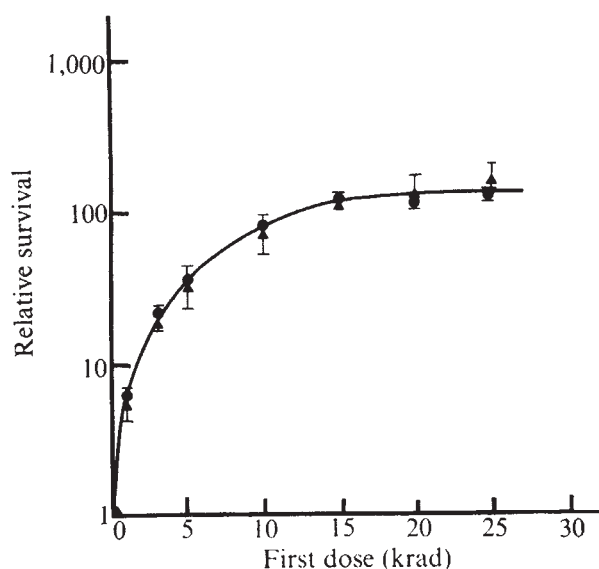


Fig. 3 Relative survival as a function of first dose given either in aerobic (●) or anoxic (▲) conditions. Cells were given various first doses in either aerobic or anoxic conditions followed by a second 'test' dose of 30 krad given in aerobic conditions 5 h later. Survival after the two doses was expressed as a fraction of cells surviving after the first dose. Relative survival values were then calculated by dividing the surviving fraction after the two-dose treatment by the surviving fraction after a single dose of 30 krad given in aerobic conditions.

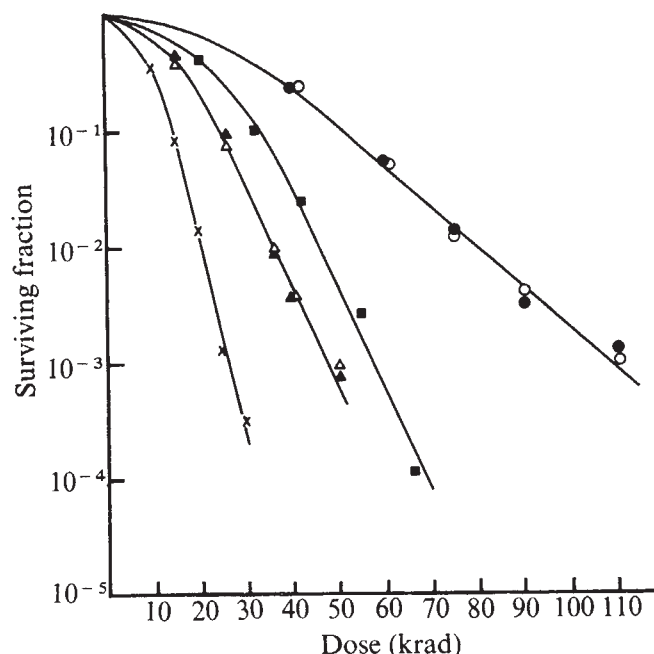


Fig. 4 Survival curves for cells given either single doses in aerobic conditions (x) and anoxic conditions (■), or a series of graded doses given 5 h after an initial dose of 15 krad given in aerobic conditions (open symbols) and in anoxic conditions (closed symbols). Second doses were given either in aerobic (▲ and △) or in anoxic conditions (● and ○). Surviving fractions of cells after two-dose treatments were normalised with respect to the surviving fraction after the first dose of 15 krad.

progressively up to 5 h after the first dose was given, the D_0 value changing from 2.8 krad for the single dose curve to 5.3 krad for the split dose curve at 5 h. The pattern of increase in D_0 with time was the same whether cells had been irradiated in aerobic or anoxic conditions (Fig. 2). The increase in resistance was also dose dependent (Fig. 3) up to about 15 krad, above which resistance reached a plateau. The dose dependence of the increase in resistance was the same whether the first doses were given in aerobic or anoxic conditions. Thus the OER for the induction of an increase in resistance was 1.0, a surprising result since the OER for cell killing was 2.2 (Table 1). This lack of an oxygen effect for the induction of resistance suggests that the initial damage causing the induction may be registered in a type N site that is in DNA. Lower OER values have been obtained for the induction of mutations than for cell killing in other cell types¹⁰⁻¹² although the values of OER for mutation induction were always somewhat larger than 1.0. In other cases quite large values of OER have been obtained for processes involving DNA such as the induction of β -D-galactosidase enzyme¹³, and the induction of prophage¹⁴.

The OER for killing *Chlamydomonas* was the same for cells given a second series of doses after an initial dose as for cells given single doses (Table 1). Figure 4 shows the survival curves for 'recovered' cells which had been given an initial conditioning dose and for cells given single doses. After an initial dose of 15 krad in aerobic conditions the surviving fraction of cells was about 8% whereas after a first dose of 15 krad given in anoxic conditions the surviving fraction was about 60%. Thus in cells given an initial dose of 15 krad in anoxic conditions only a small accumulation of sublethal damage had occurred, whereas in aerobic conditions the same dose produced the maximum amount of accumulated sublethal damage. Yet the increase in resistance was the same for the two different conditions prevailing at the time of the first dose. The increased resistance could not therefore be attributable to changes in the physical or chemical nature of the sublethally affected sites during their repair since the extent of the repair of sublethal damage in the two cases was very different. The results suggest that the increase in resistance may be attributable to an increased

capacity of cells to repair damage after a first dose has been given. It was shown in an earlier publication³ that the increased resistance could not result from more rapid repair of sublethal damage during the second irradiation, that is from a very rapid dose rate effect.

The constancy of the OER observed when the increased resistance was induced (Table 1) suggests that the balance of repair of type N and type O damage in cells remains the same. For the OER to remain constant in the more resistant cells the amounts of both enzymes (which are presumably normally present in cells) responsible for repair of the two types of lesion would have to be increased simultaneously and in proportion to the amounts normally present, so enlarging the cellular pool of both repair enzymes. It seems improbable that this could result from independent direct action of radiation on the two relevant gene sites, but could perhaps result from a 'message' passed from the initial site of radiation damage in the DNA to the two genes responsible for type O and type N enzyme synthesis. Earlier experiments¹⁵, which showed that the decrease in sensitivity of cells can be prevented by treatment of cells during the interval between doses with inhibitors of protein synthesis, lend some support to the idea that increased resistance results from the increased synthesis of repair enzymes.

In conclusion, the results presented here show that the increased resistance of cells induced by a conditioning dose of radiation depends on both time and size of the initial 'stimulating' dose given. The lack of an oxygen effect for the induction of resistance suggests that the process occurs as a result of the interaction of radiation with the DNA of the cell.

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Chemical mutagen hypersensitivity in ataxia telangiectasia

THE growth of knowledge about DNA replication and repair in bacteria has been aided by the availability of a series of mutations specifically affecting these processes. Almost invariably, defects in repair processes result in an alteration in the sensitivity of cells to ionising radiation and other mutagenic agents. Knowledge is much less advanced for animal cells, although information has been obtained using cells from patients with, for example, xeroderma pigmentosum which have provided six possible mutants in DNA repair^{1,2}. The diseases classified as chromosome breakage syndromes—ataxia telangiectasia (AT), Fanconi's anaemia and Bloom's syndrome—may also provide a rich source of DNA metabolic mutants. The frequent association of defective DNA metabolism with mutagen sensitivity has suggested an approach to the study of chromosome breakage syndromes. Finkelberg *et al.*³ have

found that Fanconi's anaemia cells are abnormally sensitive to mitomycin C (MC). We have now used this approach to determine whether cells from AT patients are potentially defective in DNA metabolism. We examined their sensitivity to actinomycin D, MC and methyl methane sulphonate (MMS), and the results indicate that AT cells are clearly more sensitive to actinomycin D and that responses to MMS and MC are more variable.

The cell lines used were obtained from skin biopsies by standard explant techniques⁴ and had been stored in a liquid nitrogen freezer before use. All had undergone less than 20 doublings after primary outgrowth. Table 1 lists the suspected genotype and other pertinent data for these cell lines. All cultures were maintained in an α medium⁵. Except where specifically noted, the α medium was supplemented with 15% foetal calf serum (FCS) (Flow Laboratories). Concentrations of nucleosides were $10 \mu\text{g ml}^{-1}$ each, of streptomycin, $100 \mu\text{g ml}^{-1}$ and of penicillin, 100 U ml^{-1} . Cultures were incubated in an atmosphere of 5% CO_2 in air at 37°C .

Cells were collected and subcultured after washing once with phosphate-buffered saline (PBS) using 0.25% trypsin (Difco) in citrate saline. The action of trypsin was stopped with 5 ml medium containing FCS.

The plating efficiency (PE) of the AT lines was somewhat lower than that of the other lines (Table 1). Although the lower PE and (we assume) associated poor growth led to rather diffuse colonies of quite varied sizes, it did not contribute to the observed mutagen sensitivity. The killing agents we used do not seem to prevent cells from attaching to Petri dishes; thus "dead" cells remain (much as a feeder layer) and contribute to a high background when high cell densities are plated. In our experiments all treatments were performed on log phase cells at an average density of

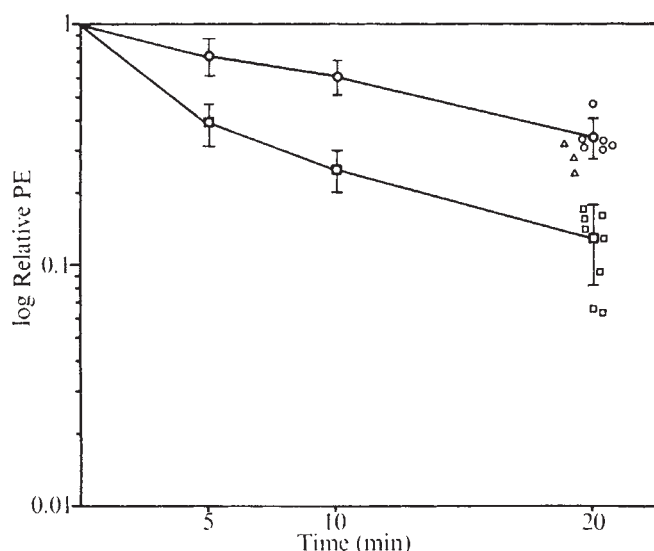


Fig. 1 Killing by actinomycin D of AT ($\square$), HET (Δ) and control ($\circ$) cell lines. Cultures to be treated were seeded in replicate at 2.5×10^5 cells per Petri dish (diameter 100 mm) 48 h before treatment. At zero time the medium was removed and replaced with medium containing actinomycin D ($1 \mu\text{g ml}^{-1}$). After prescribed incubation periods at 37°C , the mutagen-containing medium was aspirated, and the cells were washed once with medium lacking serum and then once with PBS. Treated and untreated cells were collected as described in the text and counted in a particle counter (Particle Data Inc.). Cells were diluted and five replicates at each dilution were plated in dishes (100 mm diameter) and incubated for 2 weeks before staining with 1.0% methylene blue in 70% isopropanol. With the AT cell lines many colonies were very poor, and so all scoring of colonies in control and AT lines was checked under a dissecting microscope. Only colonies containing 32 or more cells were counted. The error bars represent 95% confidence limits on 6 separate control cell lines. *Mycoplasma* testing was performed on all cultures before the experiments. All the lines used proved to be uncontaminated.

5×10^5 cells per Petri dish (100 mm diameter). With both a low PE and a maximum of approximately 5×10^5 cells per treatment point, it is impossible to carry the killing curves much beyond 2.5 logs. This difficulty could be overcome by treating larger numbers of cells. We found, however, that with smaller doses of the drugs (that is, at relatively high survival) a greater degree of reproducibility was attained. Abnormal responses were observed readily in these conditions. For example, with larger doses of MMS (30-min exposure) the average relative survival for eight independent control lines was 0.013 (s.e. 0.0057), yet in eight separate experiments with AT lines, we observed no survivors.

Cells from eight AT patients from six sibships and from three related heterozygotes (HET cells) together with six control lines (from normal subjects) were tested for sensitivity to actinomycin D. Figure 1 shows the data for the AT and control lines (with 95% confidence limits). Figure 1 includes data from two repeat experiments with two control lines and the values for the three heterozygotes, one tested twice and averaged. To indicate the variability between lines, all the data points obtained after treatment with actinomycin D are shown in Fig. 1 for 20 min of exposure.

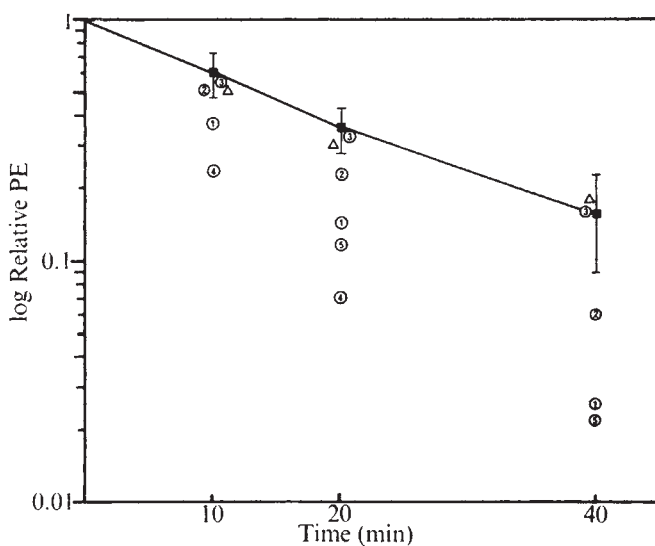


Fig. 2 Killing by MC of five AT lines with six controls (one repeated twice) and one heterozygote. Techniques were as for Fig. 1, with MC at a concentration of $1 \mu\text{g ml}^{-1}$ (Kyowa, Hakko, Kogyo Co.). Error bars are as in Fig. 1. $\blacksquare$, Controls (7); $\square$, AT194 (2); Δ , AT95 (2); $\circ$, AT97 (2); $\circ$, AT416 (1); $\circ$, AT181 (3); Δ , HET96 (1). Numbers in parentheses refer to numbers of experiments (values averaged).

Five AT cell lines, six control (one repeated twice) and one heterozygote were tested for MC sensitivity. As shown in Fig. 2, four AT lines are significantly more sensitive while the fifth, AT97, was similar to controls. Figure 3 shows MMS killing curves. In Figs 2 and 3 values are not presented for all cell lines at 5 min and in the experiments shown in Fig. 2 we did not observe any survivors for AT416 after 40 min of exposure to MC.

AT95 and AT97, which are derived from sibs, differed in their degree of sensitivity to MMS. Cells from one parent of these sibs were tested and found to be intermediate in sensitivity.

The inherent ability of a cell to survive in culture depends on its ability to correct or tolerate errors generated during normal DNA replication, recombination and/or repair. The results shown in the three figures indicate that cells derived from AT patients have a greater sensitivity than normal to various chemicals known to interact with DNA. This is not the result of their low PE: comparison of the PE and

degree of sensitivity of the AT lines (Table 1, Figs 2 and 3) shows a lack of correlation. In addition, experiments we have performed indicate that cell lines with a PE as low as 0.02 can have normal sensitivity to these chemicals.

Actinomycin D is an antibiotic that inhibits both RNA and DNA synthesis by binding directly to DNA⁶. It also has cell-cycle-specific cytotoxicity similar to that of γ radiation⁷ and produces γ -ray-like breaks in the DNA of cultured KB cells⁸. Studies with *Drosophila* have shown that besides the

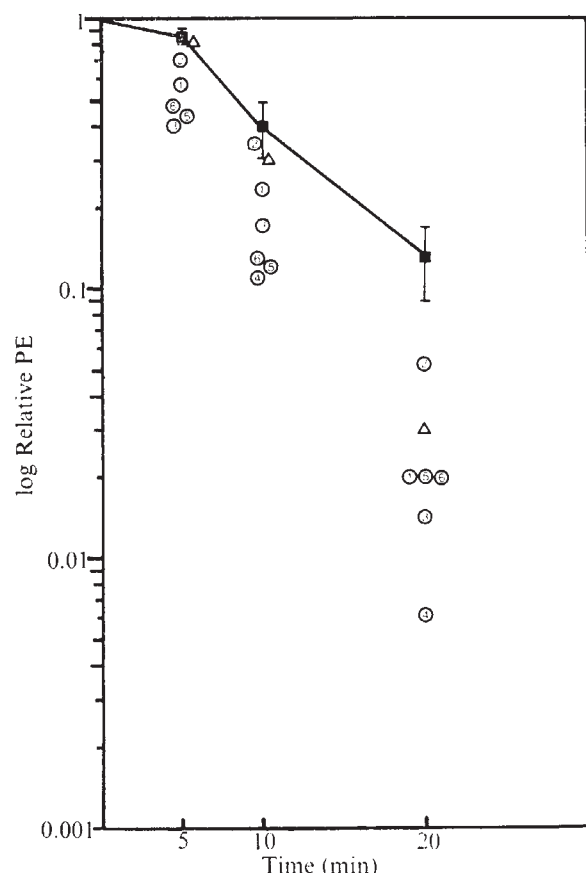


Fig. 3 Killing by MMS of six AT lines, six controls (two repeated twice) and one heterozygote. Techniques were as for Fig. 1, with MMS at a concentration of $500 \mu\text{g ml}^{-1}$ (Eastman Kodak). Error bars are as in Fig. 1. ■, Controls (7); 1, AT194 (1); 2, AT95 (2); 3, AT97 (2); 4, AT416 (1); 5, AT181 (1); 6, AT81 (1); △, HET96 (1). Numbers in parentheses refer to numbers of experiments (values averaged).

inhibitory action on RNA synthesis⁹, actinomycin D stimulates recombination specifically in the region of the centromere¹⁰. That is why we examined actinomycin D sensitivity in AT cells.

Eight AT lines from six different sibships had a significantly impaired capacity to survive low doses of the antibiotic. In addition to generalised sensitivity to actinomycin D, most of the AT lines were also sensitive to MC and MMS. Although not all cell lines have been tested with all three drugs, the apparent normal response of AT97 to MC suggests that other, possibly familial, factors influence this sensitivity. The differences in the sensitivity of AT95 and AT97 (Fig. 3) are consistent with this idea. We have observed intrafamilial differences in the degree of MC, actinomycin D and MMS sensitivity (unpublished results) and the combination of the AT genotype with a more, or less, sensitive input from one parent could produce the observed results. This explanation can be confirmed only by a study of the second parent in this sibship. It is possible that the

Table 1 Plating efficiency of the cell lines

Cell line	Sex	Age (yr)*	PE	Genotype	Relationship†
57	F	21	0.53	Control	—
62	M	35	0.35	Control	—
68	F	40	0.46	Control	—
160	F	5	0.56	Control	—
162	M	13	0.55	Control	—
192	M	2	0.67	Control	—
80	M	40	0.51	HET	Parent of 81
81	M	0.75	0.11	AT	—
82	M	14	0.40	AT	—
95	F	10	0.31	AT	Sib of 97
96	F	47	0.41	HET	Parent of 95, 97
97	M	16	0.27	AT	Sib of 95
180	M	7	0.10	AT	Sib of 181
181	M	2.5	0.19	AT	Sib of 180
193	F	31	0.39	HET	Parent of 194
194	F	9	0.098	AT	—
416	M	4	0.23	AT	—

The control lines were obtained from the Genetics Department, Hospital for Sick Children and the AT and HET lines were provided by Dr M. Thompson of the same institution. PE, average plating efficiency obtained for log phase cells at zero exposure (see Fig. 1 legend).

*Age at time of biopsy.

†Relationship between heterozygotes and patients.

variation in mutagen sensitivity and PE observed with the AT lines used is a reflection of heterogeneity in this disorder. Heterogeneity has been well documented in xeroderma pigmentosum^{1,2} and preliminary studies indicate heterogeneity at the biochemical level in AT (M. C. Paterson, personal communication).

The apparent similarities between the action of AD and γ radiation suggest that AT cells should show enhanced sensitivity to γ rays; this has been confirmed with three different AT cell lines¹¹.

It is believed that MC-induced cross links are repaired by a combination of excision repair and post-replication repair¹². The defect in the AT cells which show MC sensitivity could be in either (or both) repair systems or possibly in DNA synthesis.

Observations made with microorganisms indicate that excision repair is a highly accurate system, while post-replication repair is error prone¹². We have performed preliminary Luria-Delbrück fluctuation analysis on one AT line and found an increased frequency of spontaneous 6-thioguanine-resistant variants. This observation is consistent with an excision defect in AT cells. Such a defect has been confirmed and some AT cells seem to be deficient in γ -ray lesion-specific endonuclease¹³.

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Frequency of ultraviolet light-induced mutations is higher in xeroderma pigmentosum variant cells than in normal human cells

PATIENTS with the inherited disease, xeroderma pigmentosum (XP), are subject to multiple carcinomas of the skin on areas exposed to sunlight¹. Fibroblasts from the skin of the majority of these patients (classical XP) have been shown to be deficient in excision repair of lesions induced in DNA by ultraviolet radiation¹⁻³. One group of patients, however, has been designated 'XP variants' because, although they suffer the clinical manifestations of the disease, they carry on normal excision repair of such ultraviolet-light damage to DNA⁴⁻⁶. Lehmann *et al.*⁶ reported that cells from such XP variants are abnormally slow in converting initially low molecular weight DNA, synthesised after ultraviolet irradiation, into high molecular weight DNA similar in size to that produced in unirradiated cells. Although these authors suggest that such abnormal DNA replication might explain why such patients are susceptible to cancer of the skin, they do not propose any mechanism. If the somatic cell mutation hypothesis on the origin of cancer, first suggested by Boveri⁷, is correct one would expect the frequency of mutations induced by ultraviolet light to be higher in cells derived from skin biopsies from both classical and variant XP patients than in cells from normal persons. To test this hypothesis, we have carried out a series of quantitative investigations comparing the frequency of ultraviolet-light induced mutations to azaguanine resistance in normal human skin fibroblasts with that found in various

strains derived from classical and variant XPs. We have found that cells derived from both kinds of XP patients indeed show much higher frequencies than normal cells. The data comparing two classical XP strains with normal cells have been published^{8,9}. Here, we present data obtained with variant strain, XP4BE.

Figure 1 shows that this strain is more sensitive than normal cells to the killing effect of ultraviolet radiation. These particular cell survival data, taken from the cytotoxicity assays which accompanied each mutagenicity experiment, confirm our earlier reports⁸⁻¹¹ that XP variants are killed by lower doses of ultraviolet light than normal

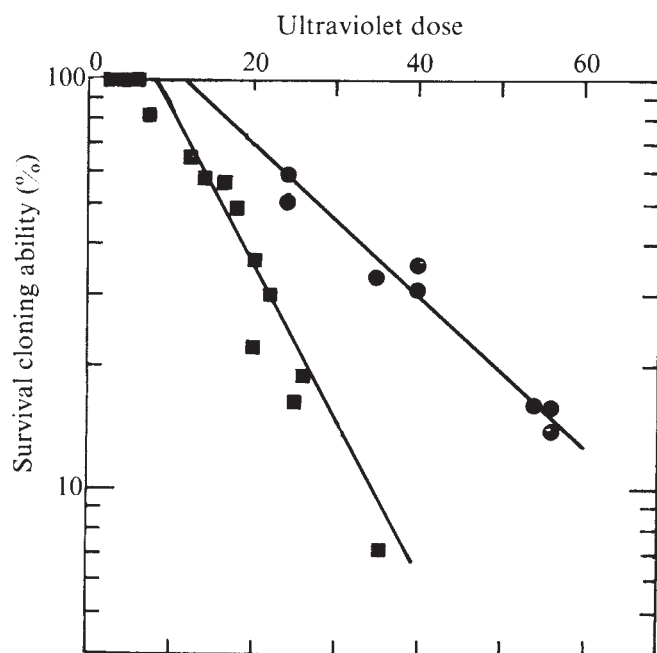


Fig. 1 Comparison of the cytotoxic effect of ultraviolet irradiation as a function of dose, in normal cells (●) with that in the variant, XP4BE (■), as determined from the percentage survival of the colony-forming ability. Survival of the cloning ability of irradiated cells divided by that of unirradiated controls is expressed as a percentage. Each symbol represents the percentage survival averaged from 12 replicate dishes of cells receiving the specified dose. Procedures for determining the cytotoxicity of ultraviolet radiation have been described^{8,10}. These survival data are taken directly from the cytotoxicity experiments accompanying each of the mutagenicity experiments shown in Fig. 2. Lines are calculated by the method of least squares.

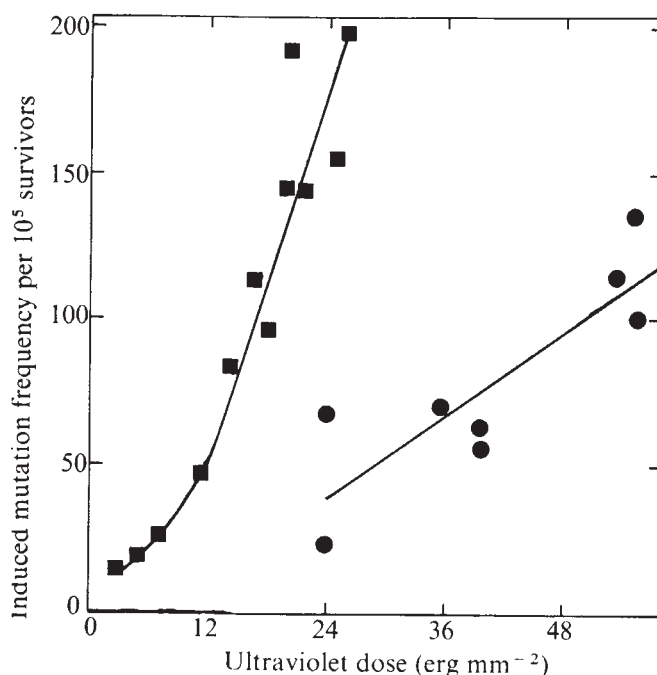


Fig. 2 Frequency of mutations to azaguanine resistance induced in normal human cells (●) and in XP4BE (■) as a function of the dose of ultraviolet radiation administered. The straight line portion of these curves was calculated by the method of least squares. For each experimental point, populations of $1-3 \times 10^6$ cells were plated in individual dishes at $0.7-18 \times 10^3$ cells per dish, allowed 10-12 h to attach, rinsed with phosphate-buffered saline, irradiated as described and given Ham's F10 medium supplemented with 15% foetal calf serum. Cells were allowed sufficient expression time to undergo three cell divisions to 'fix' the mutation and permit the necessary diluting out of pre-existing hypoxanthine (guanine) phosphoribosyl transferase enzyme. Cells were then given selection medium (F0 with 15% calf serum supplemented with 2×10^{-5} M azaguanine) three times weekly for about 28 d until macroscopic azaguanine resistant colonies appeared. Each mutation experiment was accompanied by a cytotoxicity assay to determine cell survival and a reconstruction experiment with azaguanine resistant Lesch Nyhan cells¹²⁻¹⁴ to determine the efficiency of recovery of mutants (see, for example, refs 12 and 13). The induced mutation frequencies observed in each strain have been corrected for the cloning efficiency of the particular strain to make a valid comparison between strains with different cloning efficiencies (see, for example, ref. 14). In the above experiments the cloning efficiencies ranged from 7-15% for XP4BE and from 15-25% for normal cells. The frequencies of spontaneous or pre-existing mutants in each of the experimental populations used were comparable. (This is also a requirement if a valid comparison is to be drawn between any two strains.) Fifteen independently isolated azaguanine resistant colonies of either cell strain were tested. All of them were able to form colonies in azaguanine after being cultured in the absence of this selective agent for ten or more cell doublings; 73% exhibited levels of hypoxanthine-guanine phosphoribosyltransferase activity less than 15% of normal cells (see, for example, ref. 12 for enzyme assay). A dose of 2×10^{-5} M azaguanine in our experimental conditions (serum concentration, and so on) was demonstrated to prevent an exponentially growing non-resistant population from attaining more than a 0.20 increase in cell number and to lower the survival of the colony-forming ability of the population to 0.01-0.001% of the unirradiated control.

cells even though both kinds of cells are reported to have equal rates of DNA excision repair. The corresponding mutagenicity results for these strains are shown in Fig. 2. The XP4BE strain exhibits a significantly higher than normal frequency in induced mutations per dose of ultraviolet radiation. These results provide strong support for the somatic cell mutation hypothesis on the origin of cancer.

The mechanism by which such increased mutagenicity occurs in the variants is not immediately evident. Our mutagenicity studies^{8,9}, comparing two excision-defective (classical) XP strains with normal human cells, after exposure to ultraviolet radiation, have demonstrated that the increased frequencies are directly correlated with a lack of excision and that the excision process itself does not introduce mutations. They suggest that the critical event responsible for mutations is DNA replication on a template containing unexcised lesions. Although, for a particular dose of radiation, classical XP cells have a greater number of unexcised lesions remaining in their DNA than do normal cells, this should not be the case for cells derived from XP variants. The latter, because they exhibit normal rates of excision⁴⁻⁶, should contain the same number of unexcised lesions as normal cells at any particular time after irradiation. The higher mutation frequencies observed in the variants, however, could be caused by some process related

to replication of DNA containing unexcised lesions. Trivial explanations for the observed phenomenon—such as a difference in length of cell cycle in the two strains so that less time for excision before DNA replication was available to the variant cells, or a partial synchronisation of one strain but not the other—have been ruled out (data not shown).

To investigate the question further, we carried out a series of alkaline sucrose gradient studies⁸, similar to those reported by Lehmann *et al.*⁸ to compare the size of newly synthesised DNA in XP variants with that of normal cells. The results of our sedimentation velocity studies on the size of DNA confirm their findings that in cells from XP variants, as compared with normal cells, there is some kind of defect in DNA synthesis after ultraviolet irradiation⁸. Lehmann has suggested that such DNA is synthesised from the 'inter-dimer' spaces on the irradiated DNA template and the size of the DNA is initially small because DNA synthesis is discontinuous at each dimer—that is, 'gaps' are left. Within 1–2 h, normal cells restore the DNA to the size found in unirradiated controls, but XP variants take about four times longer to effect such lengthening of the DNA molecule. Nevertheless, since the newly synthesised low molecular weight DNA seems to attain the same size as that from unirradiated cells long before the next round of DNA synthesis (data not shown), it is not obvious how a mere difference in rate of 'gap filling'¹⁵ or replication on a template which still contains unexcised dimers^{16,17} could be responsible for the increased cytotoxic or mutagenic effects of ultraviolet irradiation observed in these strains. Note, however, that the actual size of DNA in the cell is much larger than that which can be resolved with alkaline sucrose gradients (namely $\sim 1.5 \times 10^8$ daltons (ref. 6)). If the DNA in the variant cells does reach full size, one would have to assume that the slowness of the process is correlated with, or is a result of, some other defect which is ultimately responsible for both lethal and mutagenic events.

We examined the relationship between the frequency of mutations induced and the lethal effect of ultraviolet irradiation in the XP variant and compared it with that for the normal strain (data taken from Figs 1 and 2) to obtain the relationship shown in Fig. 3. The lack of overlapping points indicates that the variant actually receives a greater number of mutagenic events per lethal event than normal cells. Further work is under way to determine whether these two lines are the result of a fundamental biological difference between XP variants and normal cells, or whether the difference can be accounted for by problems inherent in comparing their different cell strains.

Nevertheless, our present results—showing that fibroblasts derived from skin biopsies from patients with greatly increased susceptibility to skin cancer caused by exposure to sunlight, exhibit frequencies of ultraviolet light-induced mutations significantly higher than normal—provide strong support for the somatic mutation hypothesis for the origin of cancer.

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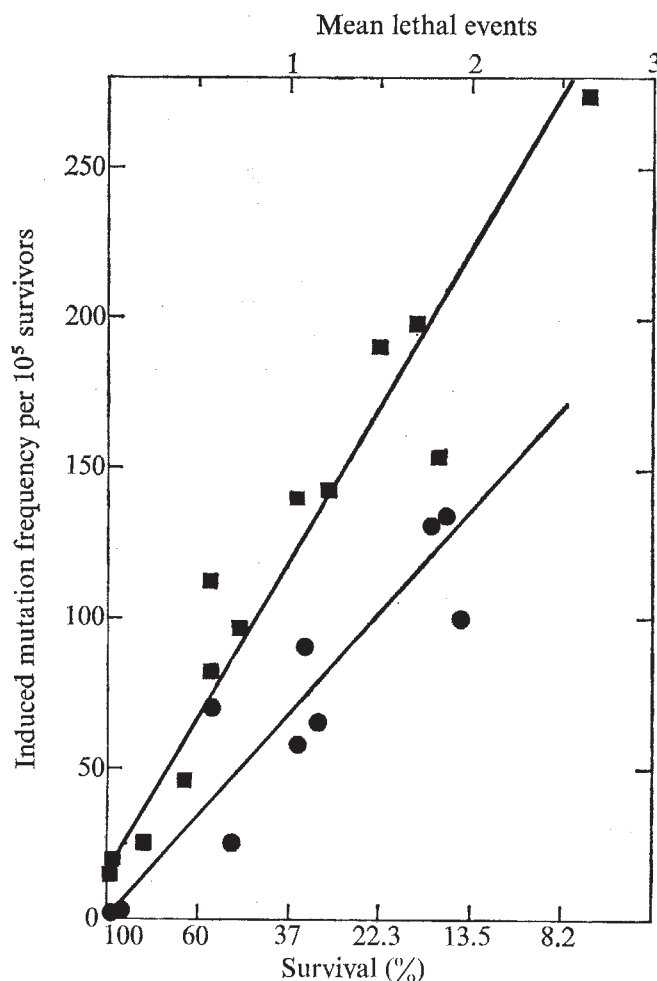
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Fig. 3 Relationship between frequency of ultraviolet light-induced mutations and its cytotoxic effect in normal human cells (●) and in variant strain, XP4BE (■). Data are taken from Figs 1 and 2. Assuming a random (Poisson) distribution of lethal events in the population, a survival of 37, 13.5 and 5% corresponds to an average number of lethal events per cell of 1, 2, and 3, respectively. The lines for each strain have been calculated by the method of least squares.



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Immunological identity of urokinase and ovarian carcinoma plasminogen activator released in tissue culture

THERE is evidence that fibrinolytic enzymes are involved in the growth of tumours. It has long been known that malignant tumours in culture lyse clotted plasma substrates¹⁻³. Davidson *et al.*⁴ found a giant-cell carcinoma of the lung to be rich in plasminogen activator and to produce it in tissue culture. Rifkin *et al.*⁵ reported an abundant release of fibrinolytic enzymes by mammalian fibroblasts transformed by DNA or RNA viruses, by chemically or virally induced mammary carcinomas and by human malignant tumours in culture. Normal fibroblasts and cultures of other normal tissues released little or no fibrinolytic enzymes. Christman and Acs⁶ found neoplastic cells, whether transformed by oncogenic viruses or by chemical agents, to release a plasminogen activator not released by normal cells. This activator was characterised as a serine protease with a molecular weight of approximately 50,000. The presence of fibrinolytic activity has been demonstrated in human ovarian tumours⁷ as well as in ascitic fluid associated with such tumours⁸. We have studied a stable plasminogen-activating enzyme, released by ovarian carcinoma in tissue culture, and we report here the apparent identity of this plasminogen activator (TA) and the activator produced by foetal kidney and present in normal urine—that is, urokinase.

The properties of the TA were examined both directly in the culture medium and after purification by affinity chromatography on para-aminobenzamidine carbodiimide coupled to CH-Sepharose 4B (Fig. 1). TA was bound firmly enough in the column and only a barely detectable amount of activity was not absorbed. Most of the protein passed through the column. After washing until no A_{280} was detectable, elution by shift of pH resulted in a sharp peak of activity. Gel filtration on Sephadex G-100 of TA purified by affinity chromatography resulted in an incomplete separation of two active peaks with molecular weights of about 35,000 and 52,000, respectively.

Neutralisation and immunodiffusion experiments were carried out with rabbit antiserum raised against Leo

urokinase (Leo Pharmaceutical, Denmark) purified by affinity chromatography and found to be monospecific¹⁰. Dilution series of purified TA were incubated with IgG from urokinase antiserum respectively with IgG from normal rabbit serum as a control (Table 1). The results demonstrate a distinct neutralisation of TA by the anti-urokinase IgG. Double diffusion in agarose gel between the concentrated culture media of six different ovarian cancers and anti-urokinase IgG resulted in distinct immunoprecipitation lines (Fig. 2). Furthermore, a complete immunological identity could be demonstrated between the

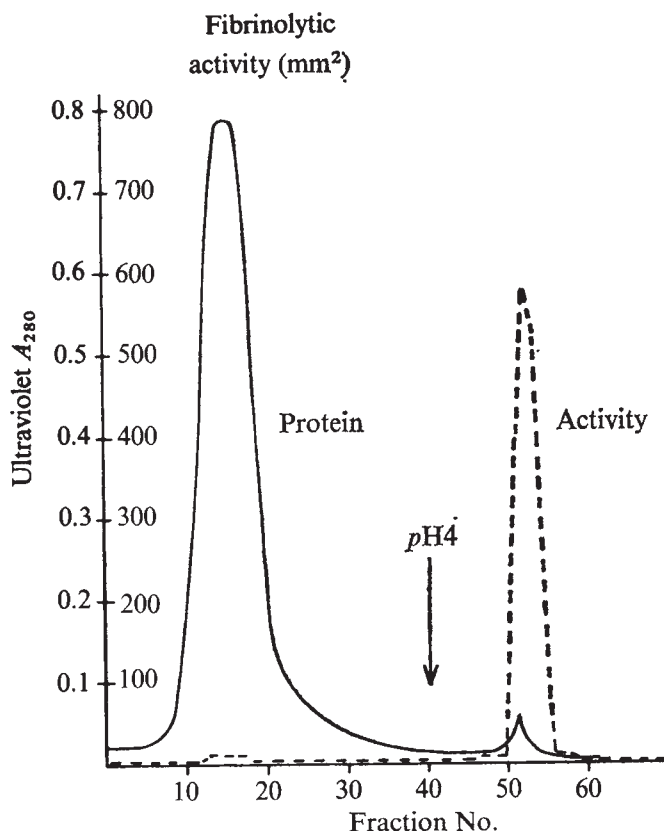


Fig. 1 Affinity chromatography of tumour culture medium on a 1.6 × 12-cm column of benzamidine-Sepharose; 150 mg *p*-aminobenzamidine (Sigma, USA) was coupled by water-soluble carbodiimide to 7.5 g CH-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala). The gel was equilibrated with 0.1 M sodium phosphate buffer, pH 7.0 and 0.4 M NaCl. Before chromatography the tissue culture medium was centrifuged, filtered and dialysed against the same buffer; 20 ml dialysed culture medium was applied (flow rate 42 ml h⁻¹). Elution was carried out by changing the buffer to 0.1 M acetate, pH 4.0, and 0.4 M NaCl. Absorbance, 280 nm. The fibrinolytic activity was tested on plasminogen-containing fibrin plates and expressed as the diameter of the lysed zones. Specimens of ovarian papillary cystadenocarcinoma were obtained at laparotomy and processed for organ and cell culture. For organ culture the specimen was divided into small fragments of about 1 mm³. These tumour explants were then placed on gelatin foam slices (Spongostan, Ferrosan, Sweden) in Petri dishes. A purely synthetic medium (Parker 199, SBL, Stockholm) was then added. Survival of the explants was checked by serial sectioning and histological examination. For cell culture the cells were prepared from the carcinoma specimen using the Borell net piston⁹. They were cultured as mixed monolayer cultures in Parker 199. The cultures were run in 5% CO₂ in air, and the medium was collected every third day and frozen. The culture media from the organ or cell cultures were constantly found to be fibrinolytically active when tested on plasminogen-containing human fibrin (Kabi, Stockholm) plates. Such activity was never observed on plasminogen-free bovine fibrin (Poviet Producten, The Netherlands) plates. When purified plasminogen was added to the plasminogen-free plates, lysis occurred, thereby showing the presence of a plasminogen activator. The highest fibrinolytic activity was found in medium of the organ cultures, which were thus the most suitable for obtaining the plasminogen-activating enzyme.

Table 1 Neutralisation of the activity of TA by anti-urokinase antibodies

Dilution	1 : 2	1 : 4	1 : 8	1 : 16
TA + normal IgG	15-18	11-14	9-10	4-5
TA + anti-urokinase IgG	Traces	0	0	0

Dilution series of purified TA incubated in proportion 2 : 1 with IgG prepared from rabbit urokinase antiserum as well as from normal rabbit serum. The fibrinolytic activity was tested on fibrin plates and expressed as the diameter of the lysed zones.

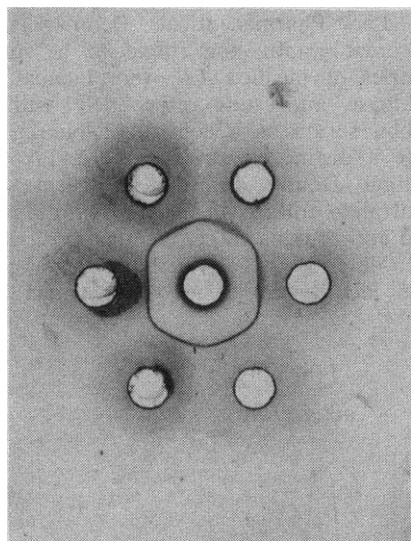


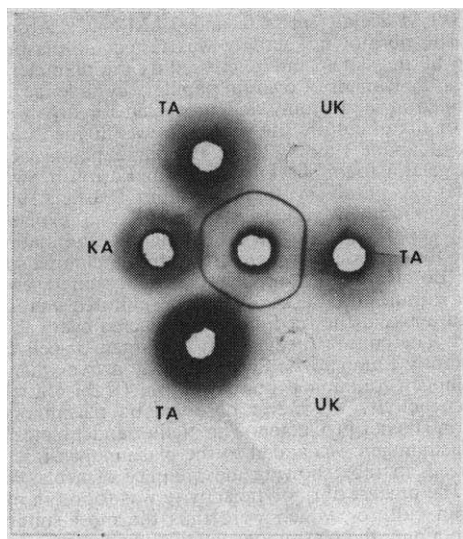
Fig. 2 Double diffusion of tumour culture medium against anti-urokinase IgG. The media from six different ovarian cancer cultures were concentrated 50–100 times on Minicon micro-concentrator cells (Amicon, The Netherlands) and placed in the outer wells and the antiserum was deposited in the central well.

tumour activator and both urinary urokinase and the activator produced by foetal kidney in culture (Fig. 3).

Figure 4 shows the inhibition of TA by di-isopropyl-fluorophosphate (DFP). The delayed inhibition of the activity in crude tumour culture medium might be explained by interference by other proteins present in the medium. There is thus evidence that TA is a serine protease. This is in agreement with Christman and Acs⁶, who found a similar inhibition by DFP of their plasminogen activator isolated from transformed hamster cell cultures. Inhibition by DFP is also a characteristic of urinary urokinase¹¹ and of the plasminogen activator released in kidney organ culture (B.A., B. Bladh and L.H., unpublished).

Release of a stable plasminogen activator in cultures of normal organs other than the kidney has been reported¹². In our experience, several normal tissues in culture produce labile activators, but only foetal kidney produces a stable activator¹³. This is supported by the observations by Kucinski *et al.*¹⁴. The plasminogen activator produced in kidney cell culture^{15,16} and kidney organ culture (B.A., B. Bladh and L.H., unpublished) is immunologically

Fig. 3 Immunological identity of urinary urokinase (UK), foetal kidney activator (KA) and ovarian cancer activator (TA). Anti-urokinase IgG in the central well and concentrated culture medium in the outer wells are as indicated.



identical to urokinase isolated from urine. We have now shown that the plasminogen activator produced by ovarian carcinoma cells is immunologically identical to urinary urokinase as well as the activator released in kidney organ or cell culture. The molecular weight distribution of TA on gel filtration suggests two molecular forms. It is known that urokinase exists in two molecular forms with molecular weights of about 31,000 and 54,000 (refs 17 and 18).

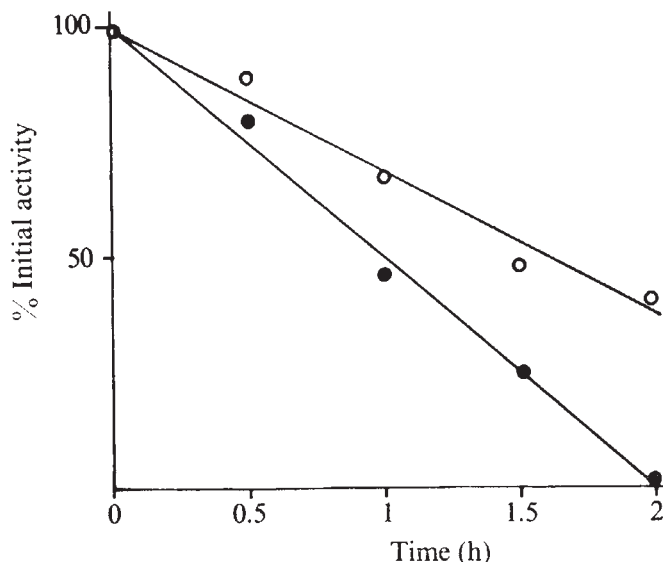


Fig. 4 Inhibition of TA by DFP. Tumour culture medium (○) and TA purified by affinity chromatography (●) were incubated at room temperature with equal amounts of DFP at a final concentration of 2.5×10^{-5} M in the reaction mixture (stock solution of 10^{-2} M DFP in propylene glycol diluted in 0.05 M Tris buffer, pH 7.8) for 0, 0.5, 1, 1.5 and 2 h. After these incubation times non-reacted DFP was removed by gel filtration on small columns filled with Sephadex G25 (Pharmacia, Uppsala) calibrated with dextran blue and K_2CrO_4 . The fractions present in the void volumes were then tested for fibrinolytic activity on fibrin plates containing plasminogen. The activity of the fractions was measured as the diameter of the lysed zone. The diameter varies linearly with the logarithm of the enzyme concentration²⁰. The activity is expressed as a percentage of initial activity.

From the data presented it can be inferred that the TA produced by ovarian carcinoma cells is very similar to, if not identical with, urokinase. In patients with malignant ovarian tumours, fibrinogen-fibrin degradation products in the blood are a common finding¹⁹, and extremely large amounts are constantly found in ascitic fluid⁸. This indicates that the TA demonstrated here is highly active *in vivo* in such patients. The role played by the TA in the survival, growth and spread of ovarian cancer can be investigated further in experimental models with urokinase antibodies.

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Hormone-like role of H-Y antigen in bovine freemartin gonad

In cattle, chorionic vascular anastomosis with a male twin modifies sexual development of a female twin fetus^{1,2}. In this condition, testosterone-dependent masculinisation of the genital tract is only moderate: the ovary is virilised to resemble a small testis in the extreme case (for reviews see refs 3 and 4). Since testosterone derived from the male twin cannot modify the ovary⁵, the cellular theory of freemartinism has been proposed^{6,7}. This theory is based on the finding that freemartins are XY/XX chimaeras with regard to not only their haemopoietic organs^{8,9} but also their gonads⁸. Indeed, the XY/XX mosaic or chimaeric gonad of mammals seems to show the definite tendency to develop as a testis. For example, so-called XX human males may actually be cryptic XY/XX mosaics¹⁰, and in certain strain combinations, a great preponderance of males is seen among the experimentally produced XY/XX mosaic mice¹¹. It has been proposed that H-Y antigen disseminated by XY gonadal cells entices neighbouring XX cells to engage in testicular organisation¹². Here we report the confirmation of this proposal on the strongly virilised foetal freemartin gonad.

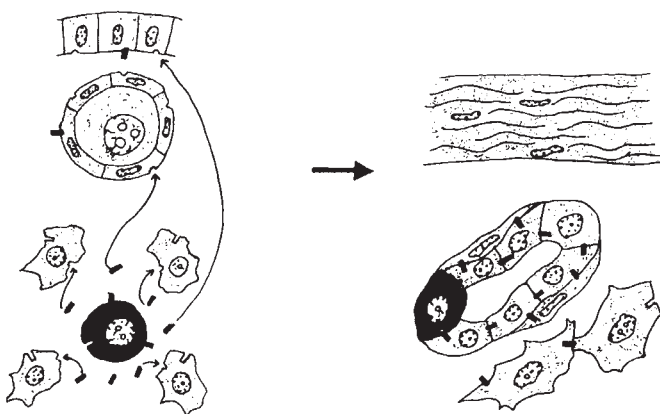


Fig. 1 Proposed virilisation mechanism of bovine freemartin gonad. Host XX germ cell and somatic elements are shaded; a donor XY cell is shown in black. We suggest that XX gonadal cells, although not possessing H-Y antigen, are equipped with specific receptor sites as well as anchorage sites for H-Y antigen (indentations on the cell surface). When coated with disseminated H-Y antigen, XX gonadal cells tend to organise testicular structure. On the left is a freemartin gonad at about 50 d of gestation: a donor XY cell that has remained quiescent allowing ovarian organisation to proceed, has just started to disseminate H-Y antigen (solid black rods). When coated with H-Y antigen, XX cells of the organised ovarian cortex are destroyed. On the right is a strongly virilised freemartin gonad at 150-175 d of gestation (Fig. 2): The ovarian cortex is replaced by a layer of hard connective tissue which now constitutes the thick tunica albuginea of the testis-like gonad. XX gonadal cells of the ovarian medulla, now coated by H-Y antigen, are forming a seminiferous tubule-like structure together with donor XY cell. Two XX cells are differentiating into Leydig cells. As most XX germ cells that were in the ovarian cortex have been destroyed, a paucity of primordial germ cells characterises the testis-like freemartin gonad (Fig. 2).

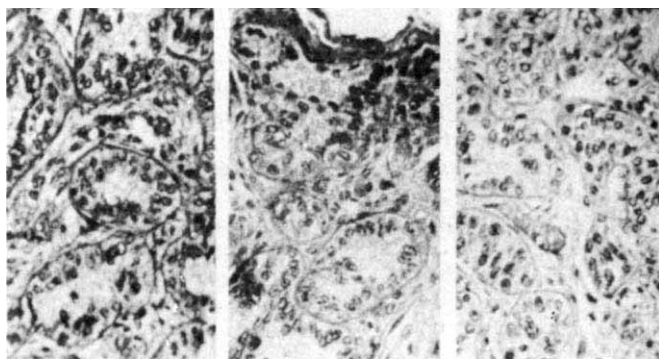


Fig. 2 Photomicrographs of histological sections from gonadal fragments of three freemartin foetuses. Lenses used were 45×6. From left to right, crown-rump lengths of foetuses were 40, 44 and 48 cm. Testis-like architecture is obvious in all three. Gonads from 48-cm freemartin foetus (right frame) were used in serological tests shown in Fig. 3 and Table 1.

Because viable cells recognise and interact with each other through their plasma membranes, cell surface components must have a key role during organogenesis^{13,14}. For this reason the phylogenetically conserved plasma membrane component recognised serologically as H-Y antigen may be the long-sought product of the testis-determining gene¹⁵. Indeed H-Y antigen is specified by a Y-chromosomal gene¹⁶, and all our studies support the proposed identity between H-Y antigen and the testis-determining gene product. Thus, we have invariably observed an association between the presence of H-Y antigen and testicular differentiation in both mice and men, regardless of phenotype¹⁷.

We propose here that XY cells from the bull twin disseminate H-Y antigen, thus coating the majority of host XX cells. H-Y antigen-coated host XX cells, together with donor XY cells, could thereby subvert development of the freemartin gonad, causing it to differentiate as a testis. According to this scheme (Fig. 1), H-Y antigen would be an enticement that XY cells extend to neighbouring XX cells. Alternatively, each H-Y antigen-bearing XY cell might come in contact with a succession of XX cells, instructing them to engage in testicular organisation. In this scheme

Table 1 Mixed MHA.HA test showing reaction of mouse H-Y antiserum with mouse sperm after absorption with cells from the gonads of foetal bovine normal female, freemartin female and male twin

Unabs. 33.0*	H-Y antiserum aliquot		
	Abs. ♀ 28.5	Abs. ♂ twin 19.0	Abs. ♀ twin† 12.0

To avoid repeated centrifugation, MHA.HA tests were performed by centrifuging sperm through a discontinuous density gradient containing wash layer and reagents in sequence^{16,28}. Gradients were established in narrow tubes by adding foetal bovine serum to each layer in a concentration adjusted to prevent intermixing. Equal volumes (0.05 ml) of a suspension containing 5-10×10⁶ BALB epididymal sperm per ml and of H-Y antiserum were reacted. The "sensitised" sperm were then placed in the gradient tube and centrifuged slowly through a wash layer and a layer containing sheep red blood cells (SRBC) sensitised with a rabbit synthetic hybrid antibody of the specificity: anti-mouse Ig/Anti-SRBC. Sperm which bound H-Y antibody (and therefore also the hybrid antibody) bound SRBC to form rosettes. Absorption of H-Y antibody in the MHA.HA test was indicated by a fall in the number of rosettes compared with the number counted after reaction of sperm with unabsorbed H-Y antiserum. MHA.HA tests were scored as coded samples by an observer who recorded the counts of rosettes and free sperm in a haemocytometer field. The values (percentages) shown above were derived from the formula: number of rosettes/number of rosettes + free sperm. Any sperm cell to which three or more SRBC had absorbed was scored as a rosette. Details of this procedure are given in ref. 16.

* Number of rosettes per 100 sperm cells.

† See Fig. 2, right frame, for cross section of freemartin gonad used in this test.

the H-Y antigen does not need to be transferred from XY cells to XX cells.

XY/XX chimaerism has always been more conspicuous in the gonad of the foetal or newborn male twin than in the gonad of the foetal or newborn freemartin. But XX cells do not feminise the testis although they may indirectly cause a marked reduction in the reproductive fitness of the bull twin¹⁸. On the other hand, the proportion of XY mitotic figures sampled from foetal freemartin gonads seldom exceeds 10%; newborn freemartin gonads often show no trace of XY cells^{7,9}. Nevertheless XX gonads containing only a minority of XY cells may be extremely virilised as noted above. It follows that observation of a substantial amount of H-Y antigen in strongly virilised freemartin gonads would go beyond confirmation of chimaerism, and would suggest that a few XY programmed effector cells can disseminate enough H-Y antigen (or act otherwise) to influence the development of host XX cells.

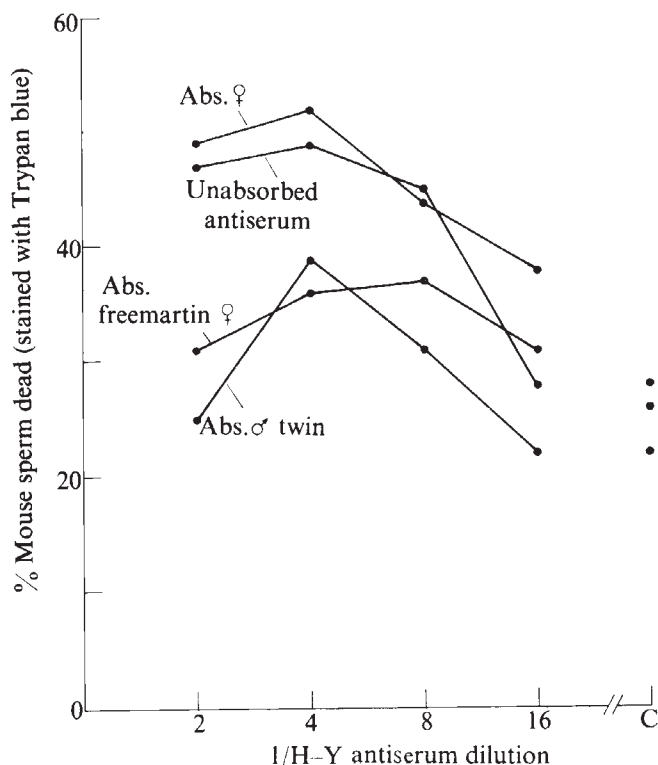


Fig. 3 Cytotoxicity test on mouse sperm with mouse H-Y antiserum after absorption with gonadal cells from foetal bovine female, freemartin and male twin. Abs. denotes absorption with cells from the subject indicated. C denotes control (complement included, antiserum omitted). H-Y antisera were raised in B6 female mice by weekly intraperitoneal injections of spleen cells from B6 males. Absorption was accomplished by suspending foetal gonad cells in small tubes containing 0.15 ml of H-Y antiserum diluted one half (one part cells : five parts antiserum) and allowing the suspension to stand for 30 min on ice. After absorption the tubes were centrifuged and each supernatant (antiserum) was removed and divided into aliquots for use in both cytotoxicity and mixed haemadsorption-hybrid antibody (MHA.HA) tests. The cytotoxicity test was performed with BALB epididymal sperm according to the method of Goldberg *et al.*²⁷. Equal volumes (0.05 ml) of H-Y antiserum (serially diluted 1/2 to 1/16), sperm (5×10^6 per ml), and absorbed rabbit serum (complement source) diluted 1/15, were incubated at 37°C for 50 min. Trypan blue dye (0.1 ml) was added during the last 10 min of incubation to stain dead sperm, and the suspensions were read as coded samples in a haemocytometer. The decrease in cytotoxic titre shown after absorption with freemartin and male twin gonadal cells indicates that these cells contain H-Y antigen. H-Y antisera used in this test were also used in the MHA.HA test shown in Table 1. A cross section of a fragment from the foetal freemartin gonad used in this test is shown in the right frame of Fig. 2. Details of the sperm cytotoxicity test are given in ref. 16.

After ascertaining that normal bovine foetal ovaries do not express H-Y antigen whereas H-Y antigen is prominent in bovine foetal testes, we collected freemartin foetuses of 40–48 cm crown–rump length (150–175 d of gestation, when the freemartin ovarian medulla undergoes masculine reorganisation^{19,20}). Three freemartin foetuses were chosen for serological determination of H-Y antigen expression, because histological examination of fragments from their gonads revealed strongly virilised testis-like architecture (Fig. 2). The results of our cytotoxicity and mixed haemadsorption-hybrid antibody tests on one of them are shown as examples in Fig. 3 and Table 1. Gonads from all these freemartins typed unequivocally and equally H-Y+.

In avian species, the heterogametic (ZW) female sex possesses W-linked histocompatibility antigen²¹, and homology has been demonstrated between H-W antigen and mammalian H-Y antigen²². According to our hypothesis, a ZW/ZZ mosaic gonad of avian species should be organised into an ovary. This is true for chimaeric heterosexual twins of chicken and duck²³: the testis of a male twin is modified to resemble an ovary. Avian primordial germ cells utilise the vascular route for their migration²⁴. Thus, gonadal chimaerism is a certainty in heterosexual twins.

It should be remembered that certain constraints are apparently imposed on a hormone-like action of H-Y antigen in many mammalian species. For example, in marmoset monkeys, the apparently mosaic gonad of chimaeric female twins develops into a functional ovary²⁵. Even in the mouse, a great preponderance of males among the XY/XX mosaics occurs only in certain strain combinations¹¹. Maybe XY gonadal cells of the marmoset-type mammalian species as well as of some strains of the mouse do not disseminate enough H-Y antigen. Conversely, the complex locus for major histocompatibility (MHC) antigens might be a principal source of the constraints. H-Y antigen seem to coexist with MHC antigens on the plasma membrane²⁶. Perhaps in the marmoset-type species a genetic difference at the MHC locus precludes effective transfer of H-Y antigen from XY to XX cells. The same might be true for some strain combinations of the mouse. Even in cattle, freemartin gonads exhibit divergent degrees of virilisation. These are problems for future studies.

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Induction of specific proteins in hyphae of *Achlya ambisexualis* by the steroid hormone antheridiol

SEXUAL differentiation in the male and female strains of the water mould *Achlya ambisexualis* occurs through a series of stages mediated by steroid hormones¹. The species-specific and sex-specific hormones, antheridiol and oogoniol², initiate the development of the male and female sex organs, respectively. Because antheridiol induction depends on RNA and protein synthesis³ and steroid hormones are thought to influence the synthesis of specific proteins in the target cells⁴, we have investigated the pattern of proteins synthesised in the male strain of *A. ambisexualis* after administration of antheridiol. We found an 'induced protein' with a molecular weight of 69,000. It was detectable 1 h after addition of the hormone. This is analogous to the effect of oestradiol on protein synthesis in the uterus of immature rats^{5,6}.

Initial experiments were carried out with total unlabelled protein obtained from hyphae treated for 3 h with antheridiol. Antheridia were clearly recognisable in these hyphae. After extraction and solubilisation of proteins, they were resolved on a sodium dodecyl sulphate (SDS)-polyacrylamide gel and stained. The pattern of proteins was scanned at 600 nm and recorded (Fig. 1a). This pattern did not differ significantly from that of the proteins obtained from uninduced hyphae (Fig. 1b). Pre-existing proteins probably mask the proteins synthesised in response to antheridiol, which are only a small percentage of the total protein present.

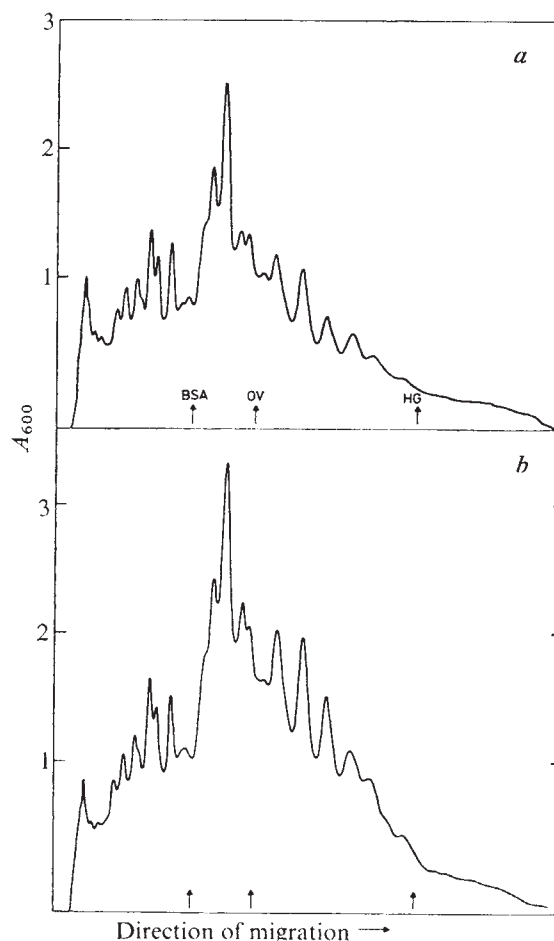
Fig. 1 SDS-polyacrylamide gel electrophoresis of total protein extracted from *A. ambisexualis* after 3 h of treatment with antheridiol (a) and from uninduced control mycelium (b). Cultures of *A. ambisexualis* E87 were maintained on agar plates of production medium¹¹. A plug of 3 mm diameter was cut out of the agar plate and incubated in 4 ml for 2 d at 25 °C. Partially purified antheridiol solution (from Dr A. Barksdale, New York Botanical Garden, 50 µl, 0.4 mg ml⁻¹, 30,000 U ml⁻¹ in 25% methanol^{12,13}) was then added to the radially growing mycelium. Three hours after addition of antheridiol, growth was stopped by chilling, mycelium was collected by centrifugation for 10 min at 10,000g and washed twice with cold distilled water. The mycelium was then resuspended in 10 ml of 50 mM Na phosphate buffer, pH 7.2, and homogenised in a Willems-Polytron homogeniser at setting 7 for 2 min at 2 °C. SDS was added to 2% and the homogenate was boiled for 1 min. After removal of insoluble material by centrifugation for 10 min at 10,000g, the proteins were precipitated with 0.1 volume of 50% trichloroacetic acid washed with 5 ml of ethanol, ethanol-ether (1:1) and ether, dried and redissolved in 10 mM Na phosphate containing 2% SDS. Electrophoresis was then carried out on 7.5% SDS-polyacrylamide gels for 4.5 h and 10 mA per gel¹⁴. Gels were stained with Coomassie blue, destained electrophoretically and scanned at 600 nm with a Gilford gel scanner. A parallel gel contained the molecular weight markers bovine serum albumin (68,000), ovalbumin (43,000) and haemoglobin (15,500).

Double label experiments were done to detect proteins synthesised when antheridiol was administered. *A. ambisexualis* was treated with antheridiol for various times and the proteins synthesised at different stages of induction were pulse labelled with ³H-leucine. After solubilisation of hyphal proteins, ³H-labelled proteins were mixed with ¹⁴C-leucine-pulse-labelled proteins obtained by the same process from uninduced control hyphae. This mixture of proteins containing ³H-leucine and ¹⁴C-leucine was then electrophoresed on SDS-polyacrylamide gels and the patterns of radioactivity were determined.

This procedure was carried out with ³H-leucine-labelled proteins synthesised in a 20-min pulse after addition of antheridiol to hyphae for 0, 0.5, 1, 2, 3, 5 and 7 h and ¹⁴C-leucine-labelled proteins from uninduced mycelium. The gels were sliced and the ³H and ¹⁴C radioactivity in each fraction was determined. To minimise statistical variation, the ratio of ³H to ¹⁴C in each gel slice was standardised by summing the individual ratios of a gel, calculating the mean of the individual fractions and dividing each ratio by the mean ratio. The deviations of the individual standardised ratios [(*R_i/R*) - 1] were calculated and plotted (Fig. 2) as a function of the gel slice number as described by Carroll *et al.*⁷. (*R_i*, individual slice ratio, *R* = *R_i*/*n*, the mean ratio, *R_i/R* the standardised ratio.) The last twenty slices containing only a small fraction of the labelled proteins were not included because ¹⁴C counts were too low to give a reliable ratio.

No appreciable deviation from (*R_i/R*) - 1 = 0 was observed in Fig. 2a. This mixture of uninduced proteins labelled with ¹⁴C-leucine and ³H-leucine for 20 min served as a control for labelling, extraction and counting. The pattern observed in mycelium induced for 30 min (Fig. 2b) showed no single induced protein although larger proteins seemed to be synthesised preferentially.

One hour after addition of antheridiol (Fig. 2c), a pre-dominant peak of protein synthesised in induced mycelium



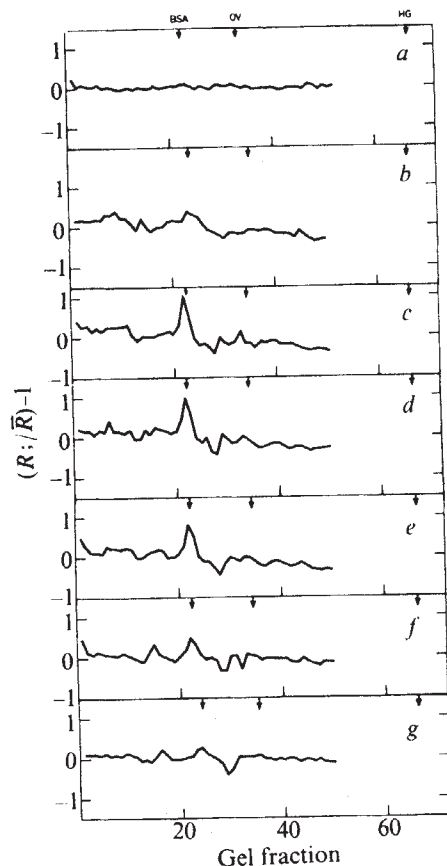


Fig. 2 SDS-gel electrophoresis of proteins extracted from total mycelium after 20 min of labelling with L- ^3H -leucine ($50 \mu\text{Ci ml}^{-1}$ 54 Ci mmol^{-1}). Labelling was preceded by treatment with antheridiol for 0(a); 0.5(b); 1(c); 2(d); 3(e); 5(f) and 7 h(g). The mycelium was cultured and induced with antheridiol, the proteins were extracted as described in Fig. 1 and more than 95% of the labelled proteins were solubilised. The ^3H -labelled proteins were mixed with ^{14}C -leucine-labelled proteins at a ratio of ^3H to ^{14}C radioactivity of approximately 5. The ^{14}C -leucine-labelled proteins were obtained from an uninduced culture labelled for 20 min with L- ^{14}C -leucine ($1.25 \mu\text{Ci ml}^{-1}$, $324 \text{ mCi mmol}^{-1}$). Electrophoresis was carried out as described for Fig. 1. Gels were sliced and ^3H and ^{14}C radioactivity in each fraction was determined by liquid scintillation counting in 0.5 ml of NCS solubiliser and 10 ml of toluene-based scintillation fluid. The ^3H and ^{14}C counts obtained simultaneously from each fraction were used to calculate the ratios as described in the text. The standardised ratios were calculated to minimise statistical variation. The three marker proteins bovine serum albumin (BSA), ovalbumin (OV) and haemoglobin (HG) were run in a parallel gel. They are indicated by arrows.

became apparent. This protein had a molecular weight of approximately 69,000 and could be recognised clearly in the gel pattern of newly synthesised proteins (Fig. 3a). Comparison with the proteins in control hyphae (Fig. 3b) revealed a pronounced shift in the pattern of protein synthesis towards the 69,000 molecular weight protein. This protein also accounted for the main difference in protein synthesis between induced and uninduced mycelium 2 and 3 h after addition of hormone (Fig. 2d and e). The most abundant protein (or proteins) migrating at about 55,000 molecular weight (observed in Fig. 1, which represents the steady state population of proteins, as well as in Fig. 3b where pulse labelled proteins are shown) was synthesised to a lesser extent in induced mycelium. This reduction in synthesis was reflected in the decreased $(R_i/R)-1$ at about fraction 28.

When the time of treatment with antheridiol was increased to 5 and 7 h (Fig. 2f and g) before the 20-min pulse labelling, the synthesis of the 69,000 molecular weight protein was reduced. The pattern of proteins synthesised 7 h after induction resembled that of the uninduced mycelium. There was still decreased production of the protein(s) migrating at about

55,000 molecular weight, however. The decrease in synthesis of the 69,000 molecular weight protein after 7 h was not due to exhaustion of antheridiol in the medium since this medium still had the biological activity to induce antheridia when added to previously uninduced mycelium.

Electron microscopic and enzymatic studies^{8,9} have suggested that the enzyme cellulase, concentrated in vesicles at the site of formation of antheridia and responsible for localised cell wall softening, is released into the culture medium. This led us to identify the proteins which are excreted into the culture medium after induction. Figure 4 shows the radioactive proteins that can be found in the medium after hormone induction for 0, 0.5, 1, 3 and 7 h followed by labelling for 20 min with ^3H -leucine. These proteins comprise approximately 1% of total labelled proteins. Uninduced mycelium (Fig. 4a) shows a broad distribution of labelled, excreted proteins.

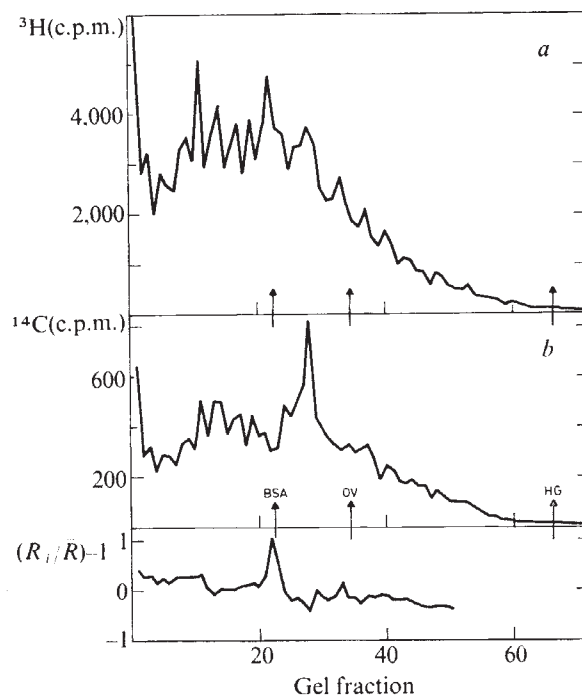


Fig. 3 SDS-polyacrylamide gel electrophoresis of proteins extracted from mycelium labelled for 20 min after 1 h of treatment with antheridiol as described for Fig. 2c. The ^3H -labelled proteins obtained from induced mycelium (a) and the ^{14}C -labelled proteins obtained from the uninduced control mycelium (b) were coelectrophoresed and the radioactivity patterns obtained simultaneously were plotted on separate panels. The standardised ratios were calculated as described in the text.

Mycelium induced for 0.5 and 1 h showed a predominant peak of excreted protein migrating at 40,000 molecular weight (Fig. 4b and c). In addition to this protein, three peaks of excreted protein migrating at 29,000, 64,000 and 88,000 molecular weight were observed after 1 h. Increasing the labelling time to 3 and 7 h (Fig. 4d and e) resulted in excretion of a complex pattern of proteins, different from that found inside the mycelium and therefore not attributable to cell lysis.

Although proteins made by *A. ambisexualis* in response to antheridiol have not been isolated and identified, this report shows that the addition of antheridiol results in the synthesis of specific proteins. The labelling protocol in these experiments (a 20-min pulse label) restricts the analysis to those proteins which are synthesised most rapidly. A protein of molecular weight 69,000 is made preferentially during the first few hours of induction. It is first synthesised 0.5–1 h after addition of hormone and precedes the microscopic appearance of antheridial initials¹⁰ which can be observed about 1 h later. After 3 h the fraction of the total protein synthesis which this protein comprises decreases again. This protein was not detected in the culture medium, but because of the labelling procedure used

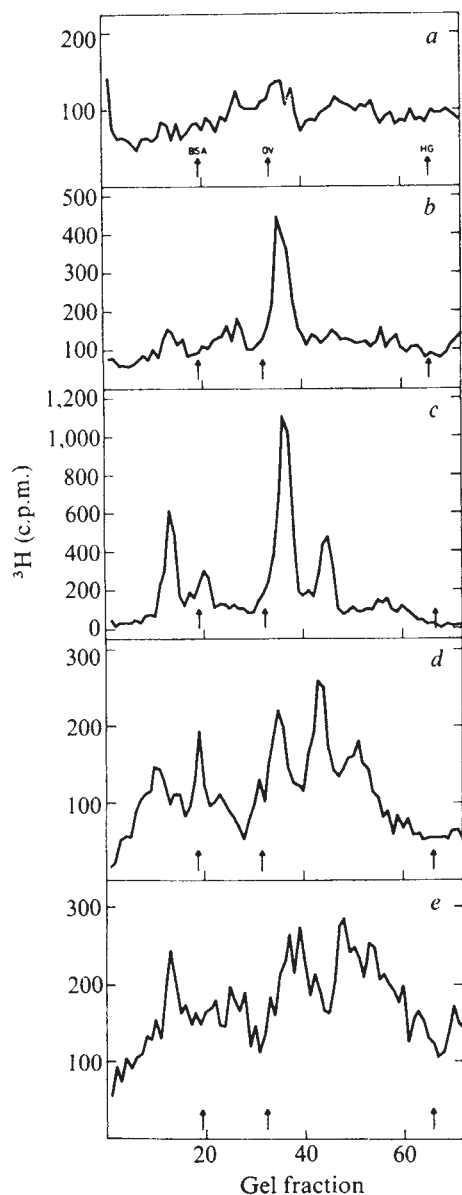


Fig. 4 SDS-polyacrylamide gel electrophoresis of proteins excreted by mycelium treated with antheridiol for 0(a); 0.5(b); 1(c); 3(d) and 7(e) h and then labelled for 20 min with L-4,5- ^3H -leucine ($50 \mu\text{Ci ml}^{-1}$, 54 Ci mmol^{-1}) as described in Fig. 2. After removal of the mycelium from the culture medium by centrifugation for 10 min at $10,000g$, the supernatant was centrifuged again at $10,000g$ for 10 min. The second supernatant was mixed with 0.1 volume of 50% cold trichloroacetic acid and the precipitated proteins were washed with ethanol, ethanol-ether (1 : 1) and ether. The washed proteins were resuspended in 10 mM Na phosphate, pH 7.2, and 2% SDS, electrophoresed and counted as described for Figs 1 and 2.

only proteins synthesised and exported rapidly would be found. This protein could have a role similar to that of the 'induced protein' found in immature rat uterus after induction by oestradiol.

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Bicyclic phosphorus esters that are potent convulsants and GABA antagonists

A SERIES of bicyclic organophosphorus (PTBO) compounds (Fig. 1) described by Bellet and Casida¹ were found to produce seizures and death within minutes of their injection into mice. In spite of the chemical resemblance of these substances to anticholinesterase agents, doses of the PTBO compounds many times those necessary to produce death had no effect on brain cholinesterase activity¹, and the mechanism of their convulsant action remained obscure. We have, therefore, investigated the action of ethyl, isopropyl and pentyl PTBO compounds as putative antagonists both of the depressant action of γ -aminobutyrate (GABA) on single neurones in the rat brain and of the depolarising action of GABA on the isolated rat superior cervical ganglion. Our results suggest that the convulsant action of the PTBO compounds may be related to their ability to antagonise the actions of GABA released as an inhibitory neurotransmitter² at synapses in the brain.

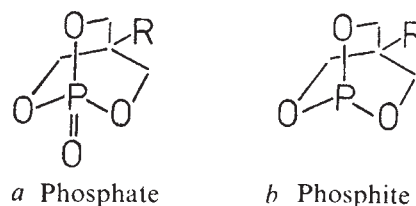


Fig. 1 Structure of bicyclic phosphorus esters. *a*, 4-(R)-1-phospha 2,6,7-trioxabicyclo(2,2,2)octane-1-oxide ((R)PTBO); *b*, 4-(R)-1-phospha 2,6,7-trioxabicyclo(2,2,2)octane. The compounds investigated had R = isopropyl (IPTBO), R = ethyl (EPTBO and ethyl phosphite) and R = pentyl (PPTBO).

The compounds were synthesised according to the method of Wadsworth and Emmons³, and then tested for convulsant activity in mice. They were also compared with the known GABA antagonists bicuculline⁴ and picrotoxin⁵. The isopropyl compound (IPTBO) was the most potent of the PTBO series and was approximately equipotent with bicuculline on a molar basis. The ethyl phosphate compound (EPTBO) had one-tenth of the potency of IPTBO and was approximately equipotent with picrotoxin whereas the pentyl compound (PPTBO) and the ethyl phosphite had less than one-hundredth the potency of IPTBO. Our estimates of the potency of IPTBO and EPTBO were in close agreement with those of Bellet and Casida¹. All six substances produced the same pattern of convulsions consisting of rapid clonic jerks, leading to tonic extension with larger doses. The similarity of the gross actions of all these convulsants suggested that it would be worth while to examine the bicyclic phosphorus esters for GABA antagonist activity, particularly as IPTBO was as potent a convulsant as bicuculline.

The central actions of the PTBO compounds were investigated in urethane anaesthetised adult rats, using techniques for single neurone recording and microiontophoretic application of drugs as described previously⁶. All single

neurone records were extracellular and taken from neurones in the medulla, some of which were physiologically identified as belonging to the cuneate nucleus⁷. In our first series of experiments attempts were made to apply EPTBO or IPTBO by microiontophoresis from seven-barrelled micropipettes. Solutions (10 mM) were prepared in distilled water, both with and without 150 mM NaCl and both positive and negative expelling currents up to 200 nA were used. No discernible effect was seen on the activity of 10 neurones studied or on the responses of these neurones to the depressant actions of GABA and glycine. It would not be predicted that these compounds would be ionised in solution and we therefore concluded that it would not be possible to expel them or control their diffusional efflux. Accordingly, a second series of experiments was started in which GABA was administered by microiontophoresis and the PTBO compounds were injected intravenously. Most of these experiments were performed using IPTBO, the most potent convulsant, and in 8 out of 11 trials attenuation of the depressant action of GABA was seen with subsequent recovery. An example of an experiment of this type is shown in Fig. 2. EPTBO (4 trials) and PPTBO (2 trials) also attenuated the action of GABA when given intravenously, although much higher doses (that is, in the ratio of their convulsant potencies) had to be given. No evidence of antagonism was seen with any of these substances until they were given in a dose sufficient to produce EEG signs of convulsive activity, although these doses were smaller than those needed to produce myoclonus.

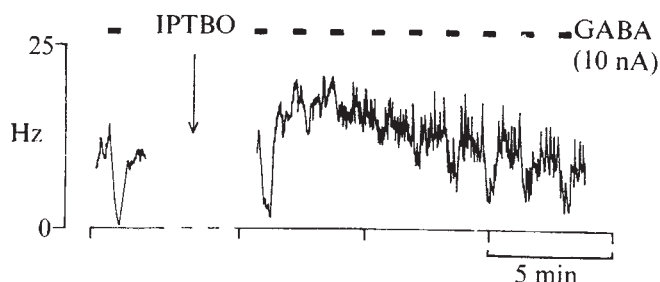


Fig. 2 Analogue rate meter recording of the spontaneous firing of a single neurone in the rat medulla (vertical scale firing rate in Hz, horizontal scale time, 5 min per division). GABA was applied microiontophoretically with a current of 10 nA for 30 s at regular intervals of 60 s determined by a digitimer. At the arrow 40 μ g (2×10^{-7} mol) IPTBO was injected into one jugular vein and applications of GABA following this injection were seen to be less effective. Within 10 min of the injection a substantial recovery was seen and this was a consistent observation in other similar experiments. Note the increase in burst firing by this neurone after IPTBO (shown by a less regular ratemeter trace) which coincided with the start of ictal spiking in the EEG. Action potential size was monitored during this experiment and remained effectively constant, suggesting that this result was not attributable to relative movement of the micropipette tip and the neurone. No myoclonus or cardiovascular correlates of seizures were seen.

In six experiments glycine was applied alternately with GABA and intravenous IPTBO was then found to attenuate the depressant action of both amino acids, although glycine seemed to be less affected than GABA on each occasion. It is therefore possible to conclude that IPTBO is a consistent but not a selective GABA antagonist when administered intravenously.

Where microiontophoresis is not practicable, more controllable experiments on putative GABA antagonists can be performed *in vitro* in a preparation such as the isolated superior cervical ganglion of the rat. GABA depolarises ganglionic neurones by an action at receptors comparable in both ligand specificity and ionic dependence to receptors for GABA on central neurones^{8,9}. This action of GABA in ganglia can be readily detected as a change in potential⁸⁻¹⁰. Ganglia were excised from Wistar rats under urethane

anaesthesia, desheathed and suspended vertically between the two non-depolarisable Ag^+/AgCl electrodes placed in contact with the postganglionic trunk and ganglion body. The tissue was superfused at 1 ml min⁻¹ with Krebs' solution containing hyoscine (2.6 μ M) at 25 °C and the potential difference across the electrodes monitored on a Servoscribe 1s recorder.

The addition of any of the four PTBO derivatives to the Krebs' solution superfusing the ganglion antagonised the depolarising action of GABA. The effect was dose dependent. Mean log dose-responses curves for GABA from five ganglia obtained in the absence and presence of 5.2, 52 and 156 μ M IPTBO are shown in Fig. 3. The presence of IPTBO shifted the curve to the right, a greater shift occurring with increase in IPTBO concentration.

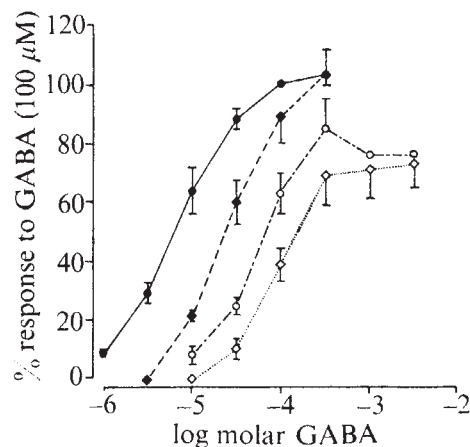


Fig. 3 Effect of IPTBO on ganglionic depolarisation produced by GABA. Ordinate: mean peak depolarisations produced during 1-min applications of GABA plotted as a percentage of the response to 100 μ M GABA in the same experiment (100 μ M GABA = 100%). Abscissa: log molar concentration of GABA. Responses were obtained in the absence (●) or presence of IPTBO at concentrations of 5.2 μ M (◆), 52 μ M (○) and 156 μ M (◇). The data were obtained from five separate ganglia. Vertical bars represent s.e.m. GABA was applied at 20 min intervals and each concentration of IPTBO was in contact with the tissue at least 15 min before subsequent applications of GABA.

Depression of the maximum response to GABA occurred at 52 and 156 μ M IPTBO, an effect which contrasts with that of bicuculline and bicuculline methochloride but compares with that of tetramethylenedisulphotetramine (TETS)^{8,9,11}. The effect of IPTBO was, however, readily reversible and, within 15 min of its removal, responses equal to control level could be obtained. IPTBO seemed to be comparable in potency with (+)-bicuculline methochloride and TETS. Equimolar amounts of the three substances depressed responses to GABA (30 μ M) by approximately the same amount. The similar potencies of (+)-bicuculline methochloride and TETS in the ganglion have already been noted¹¹. The relative potencies of the compounds can be summarised as: IPTBO=1, EPTBO=0.1, ethyl phosphite=0.03, PPTBO<0.01. These results are in close agreement with the ratio of their convulsant potencies.

The compounds seemed to be specific antagonists of the action of GABA in the ganglion. Responses to the cholinomimetic, carbachol, which also depolarises ganglia in a dose-dependent manner¹², were unaffected by the PTBO compounds when responses to GABA were blocked or considerably reduced and the action of the other ganglionic stimulants, 5-hydroxytryptamine and angiotensin, was similarly unaffected by the presence of IPTBO.

The results obtained with the compounds examined so far suggest that the potency of these compounds as GABA antagonists depends solely on the alkyl group (R) in the molecule (Fig. 1). Increasing the size of this group from

ethyl to isopropyl produces a substantial increase in potency both as a convulsant and as a GABA antagonist but a further increase in size to pentyl produces a dramatic decrease in potency. It would therefore seem that these compounds combine with a site on the subsynaptic membrane that has a lipophilic cleft of a defined size into which the alkyl group fits. The molecule can be considered as a conformationally restricted skeleton maintaining the alkyl group and the rest of the molecule in the correct three-dimensional relationship. It is interesting to note that ethyl phosphite (Fig. 1) was active both *in vivo* and *in vitro* as it has been suggested that this compound is only active after bio-oxidation to the phosphate². The ethyl phosphite, although hygroscopic, is stable to water¹³ and the observation that it is of lower potency than the corresponding phosphate may give a pointer to possible chemical substitution at the phosphorus-containing part of the molecule. Further synthesis of compounds in this group is in progress to allow a more confident interpretation of the structure-activity relationships involved. This is an important area of research as EPTBO, in sufficient concentration to convulse rats¹⁴, has been shown to be present in the smoke given off by burning fire-retarded polyurethane foams and the problem of finding an antidote to poisoning by compounds such as these can only be solved by a complete understanding of their mode of action.

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Independence of β -adrenergic and thyrotropin receptors linked to adenylate cyclase in the thyroid

CATECHOLAMINES stimulate thyroid function in several species, including cattle, man and mouse (brief review in ref. 1). As with the pituitary-derived tropic hormone, thyrotropin, an initial step in this process seems to be stimulation of adenylate cyclase associated with the thyroid cell plasma membrane, leading to increased intracellular

adenosine-3'-5' cyclic monophosphate (cyclic AMP). The response to catecholamines is mediated by a β -adrenergic receptor^{2,3} and we have examined whether, for a given membrane preparation, the response of adenylate cyclase to the β -adrenergic agonist isoproterenol can be varied independently of the response to thyrotropin. Our results indicate that the two receptor-adenylate cyclase systems function separately.

Bovine thyroid membranes were prepared as before⁴, and adenylate cyclase activity was determined in the conditions described previously⁵. The cyclic AMP formed was assayed as before⁶. The methods of Smith and Hall⁷ were used to prepare plasma membranes from the human thyroid, and to study the binding of iodinated thyrotropin.

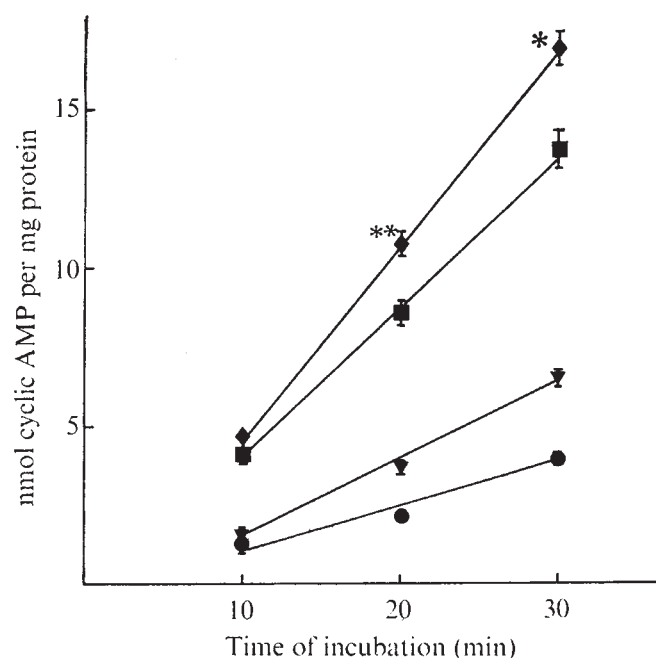


Fig. 1 Time course of stimulation of adenylate cyclase in bovine thyroid plasma membranes by isoproterenol (∇ , 2×10^{-5} M), thyrotropin ($\blacksquare$, 50 mU ml⁻¹) and a combination of the two (diamonds). Membrane preparations (10 μ g membrane protein) were added to tubes equilibrated at 37 °C, containing 2.5 mM ATP, 5 mM MgCl₂, 10 mM theophylline, 25 mM Tris-HCl, pH 7.4, 10 mM creatine phosphate, 20 μ g of crystalline rabbit muscle creatine kinase. Bovine thyrotropin (Thyrotropin, Armour) and isoproterenol were added as indicated, in a final incubation volume of 120 μ l. Results are expressed as means of quadruplicate determinations of adenylate cyclase activity $\pm$ s.e.m., each assayed for cyclic AMP in duplicate. Isoproterenol plus thyrotropin compared with thyrotropin alone: * $P < 0.05$; ** $P < 0.005$. $\bullet$, Basal level of cyclic AMP.

Figure 1 shows a time course of basal adenylate cyclase activity stimulated by thyrotropin and isoproterenol in bovine thyroid preparations. Significant stimulation by thyrotropin (50 mU ml⁻¹) and isoproterenol (2×10^{-5} M) was seen after 10 and 20 min of incubation, respectively. Simultaneous addition of both stimulators resulted in further stimulation of adenylate cyclase activity, caused in an additive manner. Because 2×10^{-5} M isoproterenol is sufficient to provoke a maximal response (Fig. 2 and ref. 3), the increased adenylate cyclase activity in the presence of thyrotropin suggests stimulation through separate systems. We examined this possibility by testing for selective inhibition of the response to the two stimulators.

1-Propriolol and polyphlorethol phosphate (PPP) were used as selective inhibitors of isoproterenol and thyrotropin stimulation respectively. 1-Propriolol inhibits both isoproterenol and thyrotropin stimulation of adenylate cyclase activity in bovine thyroid membrane preparations^{3,8}, but the concentration required to inhibit the former is several orders of magnitude lower than that needed to inhibit the

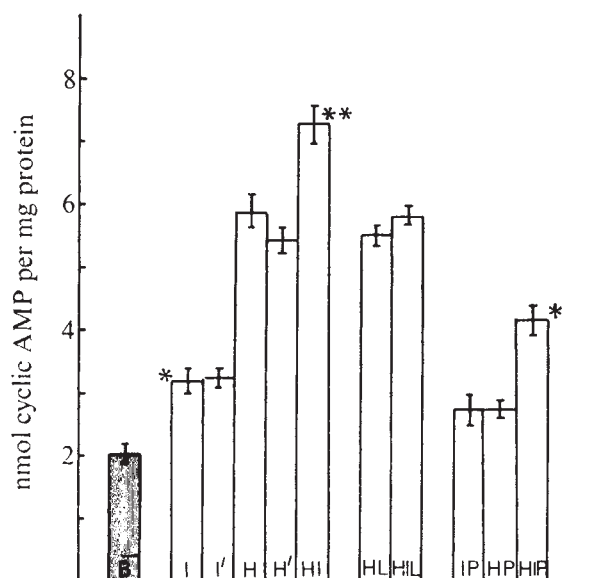


Fig. 2 Selective inhibition of isoproterenol and thyrotropin stimulation of adenylate cyclase in bovine thyroid plasma membranes. Conditions of incubation as for Fig. 1. Incubation was for 30 min and the membrane preparation used was not that used for the experiment shown in Fig. 1. Results expressed as mean of quadruplicate determinations of adenylate cyclase activity $\pm$ s.e.m., each assayed for cyclic AMP in duplicate.

The hatched column shows the basal level. I, 2×10^{-6} M isoproterenol; I', 10^{-4} M isoproterenol; H, thyrotropin (50 mU ml $^{-1}$); H', thyrotropin (100 mU ml $^{-1}$); L, 10^{-6} M L-propranolol; P, PPP (10 μ g ml $^{-1}$).

*I compared with basal, and HIP compared with HP, $P < 0.0005$.

†HI compared with H, $P < 0.0005$; IP not significantly different from I.

latter. Conversely, PPP (10 μ g ml $^{-1}$) inhibits stimulation by thyrotropin in bovine thyroid plasma membranes, but not that by isoproterenol (Fig. 2). As Fig. 2 shows, adenylate cyclase activity stimulated by the presence of both thyrotropin and isoproterenol was reduced by addition of 1-propranolol (10^{-6} M) to the activity seen in the presence of thyrotropin alone. Confirming previous results, the inhibitor was without significant effect on stimulation due to thyrotropin alone. PPP (10 μ g ml $^{-1}$) selectively reduced stimulation by thyrotropin, however, such that in the presence of both thyrotropin and isoproterenol, the response seemed to be that due to isoproterenol plus the diminished response to thyrotropin. Such selective inhibition was consistent with independence of the receptor-adenylate cyclase systems.

Table 1 Failure of isoproterenol or adrenaline to displace thyrotropin bound to human thyroid membranes

Additions	Iodinated thyrotropin bound %
None	20.3 $\pm$ 0.81
+ Thyrotropin (165 mU ml $^{-1}$)	3.7 $\pm$ 0.31
+ IgG*	11.0 $\pm$ 1.0
+ NIgG*	20.6 $\pm$ 1.7
+ Isoproterenol (10^{-6} M)	19.5 $\pm$ 0.72
(10^{-5} M)	20.1 $\pm$ 0.74
(10^{-4} M)	21.1 $\pm$ 1.26
+ Adrenaline (10^{-4} M)	22.6 $\pm$ 1.35

Incubation conditions were as described in ref. 7, except that label was added at the beginning of the 1 h of incubation at 37 °C simultaneously with unlabelled thyrotropin, isoproterenol or adrenaline. Final concentrations in the incubation tube are shown, and membranes were present at a concentration of 400 μ g membrane protein ml $^{-1}$. Results are expressed as the mean of duplicate determinations $\pm$ s.d.

*Immunoglobulin G (IgG) preparation from thyrotoxic serum ($\times 5$ concentrate). No inhibition observed with a similar preparation of IgG from control serum (NIgG).

Table 1 confirms reports that iodinated thyrotropin binds to membrane preparations from the human thyroid in a reversible manner, and can be inhibited by an immunoglobulin fraction from thyrotoxic sera $^{7-10}$. Moreover, the specific nature of binding was confirmed by the observation that neither isoproterenol, present in concentrations ranging from 10^{-6} to 10^{-4} M, nor 10^{-4} M adrenaline caused significant displacement of bound thyrotropin. This suggests that the receptor system for isoproterenol and thyrotropin are functionally disassociated in thyroid membrane preparations.

Thus, although Spaulding and Burrow 2 failed to detect additivity when studying thyrotropin and isoproterenol stimulation of intracellular cyclic AMP in slices of thyroid tissue, our results indicate that in plasma membrane preparations, receptors for thyrotropin linked to adenylate cyclase function independently from the β -adrenergic receptors.

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Different distribution pattern of 100-Å filaments in resting and locomotive leukaemia cells

Two types of filaments have been described in the cytoplasm of non-muscular cells—microfilaments (40–70 Å in diameter) and 100-Å filaments (90–110 Å). Microfilaments have been found in almost every type of animal cell 1 , and they are known to be actin-like 2 and believed to have a role in many contractile processes 1,3 . In contrast, the 100-Å filaments have been observed only in a limited number of cell types, such as tissue culture cells 2,4 , smooth 5 and skeletal 6 muscle cells, nerve cells 7,8 and tumour cells 9,10 , and are thought not to be actin-like 2,3,10,11 . The function of 100-Å filaments is not clear although they have been suggested to act in the intracellular transport of organelles in fibroblasts 4 , the axoplasmic transport in nerve cells 12,13 and the spreading of cells 3 , or to serve as a cytoskeleton 14 . Induction of 100-Å filaments by colcemide 6 and cytochalasin B and colcemide 15,16 has also been demonstrated. A clue to their function may lie in the demonstration, reported here, that the distribution of the 100-Å filaments differs in resting and locomotive cells.

We are studying the correlation between motility and ultrastructure in leukaemia cells. Our main experimental model is the undifferentiated transplantable leukaemia L 5222 (ref. 17), propagated in the inbred BD-IX rat 18 . By time-lapse cinematography, the cells of this leukaemia are recorded in two functional

states¹⁹, as resting cells which are completely spherical, and as locomotive cells which have a conspicuous extension at the rear end resembling the uropod of stimulated lymphocytes²⁰. This difference in shape makes it possible to distinguish between cells fixed at rest and in locomotion, respectively. For electron microscopy, cells were processed by double fixation (2% glutaraldehyde, 1% osmium tetroxide), ethanol dehydration, and embedding in Epon. To determine the actin- or non-actin-like nature of the filaments, L 5222 cells were glycerinated and incubated with HMM according to the procedure of Ishikawa *et al.*², and then prepared for electron microscopy. Thin sections of normal and HMM-treated cells were photographed with a Siemens 102 electron microscope.

L 5222 cells have an appreciable amount of both microfilaments and 100-Å filaments. The microfilaments bind HMM by forming characteristic arrowhead complexes, which proves their actin-like nature. On the other hand, 100-Å filaments show no reaction with HMM. Microfilaments are arranged in a fine mat-like structure beneath the cell membrane. This pattern is identical in resting and in locomotive cells. In contrast, there is a striking difference in the distribution patterns of the 100-Å filaments in the two functional states of L 5222 cells. Resting cells have thick bundles consisting of 100-Å filaments in parallel array (Fig. 1a), closely resembling the aggregates of 100-Å filaments recorded in cultures of myogenic and fibrogenic chick embryo cells^{6,16}. The large bundles can reach a diameter of several μm , and the cytoplasmic rim between cell membrane and nucleus may be filled out by them. The 100-Å filaments seem to be associated closely with mitochondria and nuclear membrane (Fig. 1b). In locomotive cells, there are no large bundles, and the 100-Å filaments are distributed singly and in small groups within the uropod-like protrusion (Fig. 2).

In preliminary studies of the motility of human leukaemia

Fig. 1 *a*, Resting L 5222 leukaemia cell with a large bundle of 100-Å filaments (arrow) ($\times 10,875$). *b*, Close association of a bundle with mitochondria (double arrow) and nuclear membrane (arrow) ($\times 25,230$).

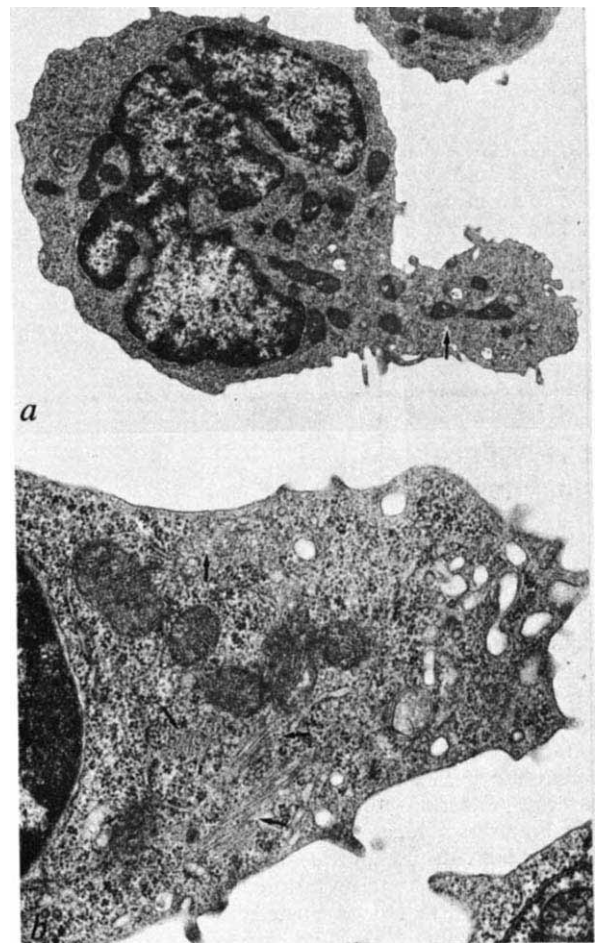
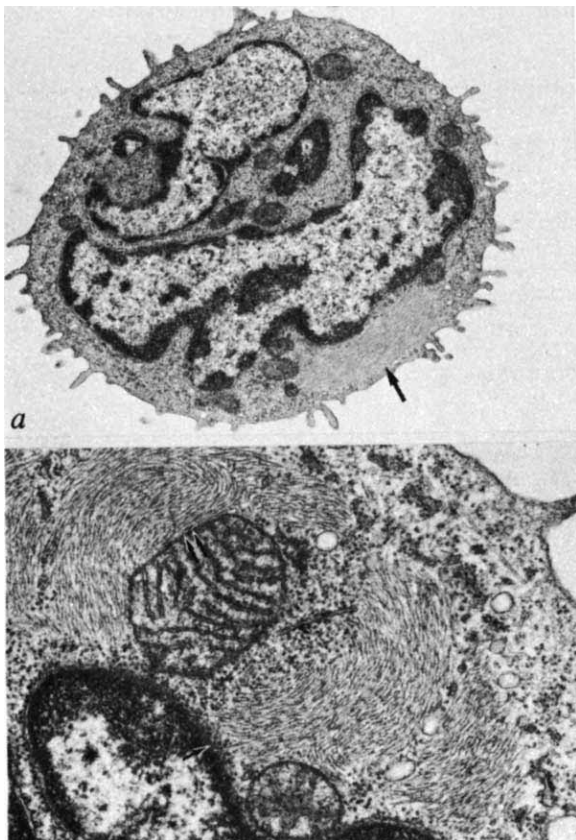


Fig. 2 *a*, Locomotive L 5222 cell with uropod-like protrusion ($\times 7,680$). *b*, Higher magnification of another uropod-like protrusion ($\times 24,000$). Small groups of 100-Å filaments are indicated by arrows.

cells, we have found a similar correlation between the arrangement of 100-Å filaments and locomotive behaviour (unpublished results). In one case of an acute myeloid leukaemia, the lack of any cellular locomotion was associated with the occurrence of huge bundles of 100-Å filaments in nuclear indentations, whereas in the highly locomotive cells of another case of human leukaemia, the 100-Å filaments were found only in small groups.

In summary, it seems that in resting leukaemia cells the non-actin-like 100-Å filaments can be arranged in large bundles, whereas with the onset of locomotion these filaments become rather loosely distributed in the cytoplasm. The significance of the different distribution pattern of 100-Å filaments in resting and locomotive leukaemia cells is unclear and needs further experimental investigation.

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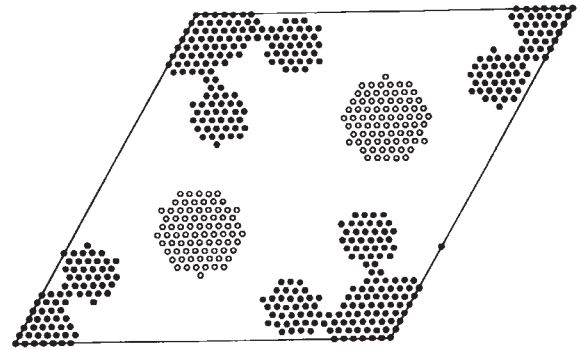


Fig. 1 Our model VI for the equatorial electron density distribution of a relaxed muscle. The model is based on Lymn's model II⁴ with the thick filament rotated by 12° and the cross bridges extended by 1 nm. The spacing between adjacent points is 1 nm and the length of a side of the unit cell is 40 nm. ●, Electron density of 20 arbitrary units; ○, 15 such units; the remainder of the cell is considered to have zero density.

Cross-bridge movement during muscle contraction

WHEN vertebrate striated muscle contracts at constant length, the 1,0 reflection decreases in intensity while the 1,1 reflection increases in intensity^{1,12}. These changes have been interpreted as arising from the movement of the cross bridges from the vicinity of the thick filaments in relaxed muscle to the vicinity of the thin filaments in the contracting state, and this movement has been thought to involve both radial and azimuthal components^{2,3}. Lymn⁴ has claimed that models with cross bridges near the thick filament do not simulate the intensity distribution of the higher order equatorial reflections of relaxed muscle, while models in which the cross bridges are extended to near the radius of the thin filaments do give good simulations. This has led him to contest the interpretation of the intensity changes given above, and to propose instead a model in which these changes are brought about by azimuthal movement alone. In this article we challenge Lymn's interpretation on several grounds.

Lymn based his conclusions on a study of five models. When we recalculated the intensities expected from his models, we noticed several discrepancies (Table 1). In his model I, Lymn's published value for the intensity of the 1,1 reflection is too high by almost a factor of two, and more importantly, in his model II, his value for the intensity of the 2,2 reflection is ten times too low. We have verified our computations where they differ from Lymn's by independently resynthesising the original structures by Fourier inversion. Lymn had rejected his model II (and all models

in which the cross bridges are near the thick filament) in preference to his models IV and V (where the cross bridges are at higher radius) because he considered that his calculated intensities of the higher orders for model II were too low to account for the pattern from resting muscle. But when our calculated intensities are compared with those observed experimentally (Table 1) it is not easy to decide between models II, IV and V as representing resting muscle, and certainly model II can no longer be dismissed. By rotating the cross bridges in Lymn's model II through 12° and extending them by only 1 nm, we generated a model VI with short cross-bridge arms (Fig. 1) for which the agreement between observed and predicted intensities is considerably better than for any of Lymn's models with cross bridges at higher radius (Table 1). We believe, however, that all these models are somewhat unrealistic because the distribution of mass between the cross bridges, the backbone of the thick filament and the thin filament does not correspond to the values determined experimentally for vertebrate striated muscle^{5,6}. Furthermore, we believe that detailed modelling experiments are premature at the moment because too little information is available concerning the number, shape and size of the cross bridges and their orientation relative to the thin filaments.

We have therefore attempted to derive a model of the equatorial density distribution directly from the measured intensities of the first five reflections by Fourier synthesis.

Table 1 Relative equatorial intensities

Model	I*	II	III	IV	V	VI	
Length of cross bridges	Long	Short	Short	Long	Long	Short	Experimental
Reflection							
1,0	100	100	100	100	100	100	100
1,1	190 (324)	47	33	30	26	39	44
2,0	47	7	3	36	4	7	9
2,1 1,2	62	2	11	12	20 (36)	9	9
3,0	16	0	18	155	35	4	12
2,2	34	21 (2)	1	11	19	10	} 20
3,1 1,3	30	0	1	23	85	19	
$I_{1,0}/I_{0,0}$	0.112	0.515	0.525	0.124	0.081	0.434	—

To avoid sampling errors intensity was calculated directly as

$$I_{hk} = (F_{hk})^2 / (h^2 + k^2 + hk)^{1/2}$$

where

$$F_{hk} = \sum_x \sum_y \rho_{xy} \exp[-2\pi i(hx + ky)]$$

and where h and k are the indices of the reflection and x and y are the fractional cell coordinates of each density point. I_{hk} was calculated for all points in the reciprocal lattice and the values for those points which superimpose on the pattern were then added. Values where Lymn's quoted intensities are significantly different from ours are underlined with Lymn's values given in parentheses. We have ignored errors of phases.

The intensity of the 0,0 reflection was calculated directly as

$$I_{0,0} = (\sum_x \sum_y \rho_{xy})^2$$

*Models I to V are described in Lymn⁴ and model VI is shown in Fig. 1.

(For consistency, we have taken Lymn's reported values given in Table 1.) We have taken the phases of the 1,0 and 1,1 reflections as positive¹. Of the possible combinations of + or - for the remaining reflections, adequate densities for the thin filaments were only obtained when the 3,0 reflection was positive. The best simulation of the relative masses of the thick and thin filaments was obtained when the 2,0, the 2,1 and the 1,2 reflections were all negative, although here the effect of phase was not so marked. The synthesis obtained is shown in Fig. 2 and is clearly in accord with models in which the cross bridges are at a radius less than that of the thin filaments. Similar results, but with lower relative thin filament densities, were obtained with other combinations of phases for the 2,0, the 2,1 and the 1,2 reflections. In these syntheses an implicit assumption of sixfold rotational symmetry has been made.

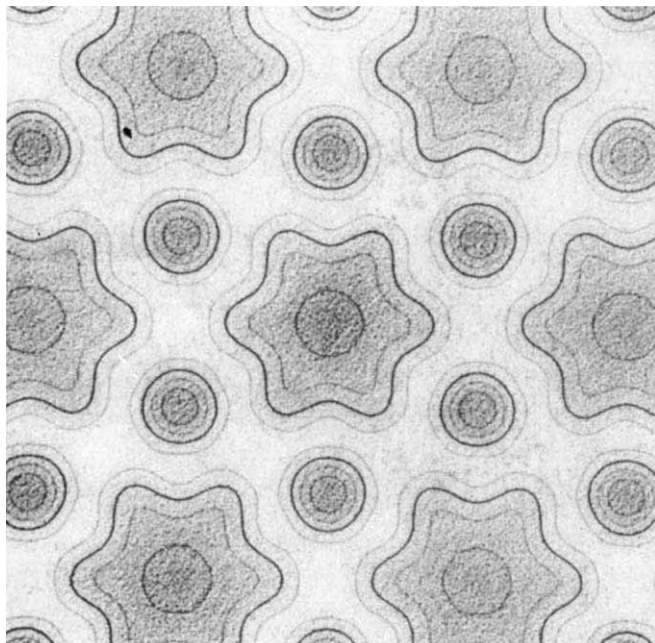


Fig. 2 A possible electron density distribution in relaxed vertebrate striated muscle at rest length calculated by a Fourier synthesis based on the intensities of the first five equatorial reflections as given by Lymn¹. For simplicity, we have assumed that reflections which lie at the same distance from the origin (and thus contribute to one observed reflection) all have the same intensity. All combinations of positive and negative phases were tried for the 2,0, 2,1, 1,2 and 3,0 reflections and for this map the phases were -, -, - and + respectively. The four contours shown are equally spaced and the density is also simulated by stippling. Because only the first five reflections were included, the synthesis is not sharp, but it is consistent with the cross bridges being in an area approximately bounded by the second lowest contour (shown in heavy outline) and so being located at a radius less than that of the thin filaments, although their exact outline is unclear.

We feel that this synthesis should be treated with some caution until the symmetry of the thick filament has been established (which will show whether the phases are all real), although it is unlikely that the radial distribution of mass about the thick filament will be much different if the equatorial projection of the thick filament does have nine-fold symmetry⁷.

But whatever its exact relation to the distribution of density in relaxed muscle, this synthesis shows that it is possible to produce a model with the cross bridges at a radius less than that of the thin filaments which simulates the observed intensities exactly. We therefore believe that Lymn's rejection of any substantial radial movement was premature because his fundamental premise—that the equatorial pattern of relaxed muscle could not adequately be simulated with models with their cross bridges near the thick filaments—was incorrect.

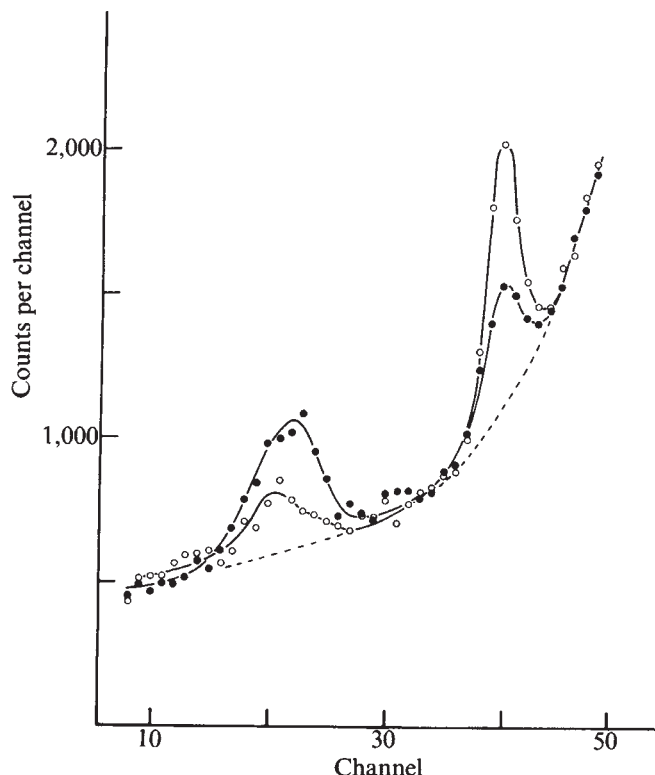


Fig. 3 Trace taken using a position sensitive counter^{8,9} of the first two equatorial reflections from a frog sartorius muscle at rest length. ○, Relaxed; ●, the same muscle contracting isometrically. The total exposure was 10 s, which was summed from several short tetani.

We have also studied the absolute change in intensity of each of the first two equatorial reflections when a muscle contracts. The intensity of the 1,0 reflection is 50% ($\pm 10\%$, s.d. on 12 muscles) weaker in muscles during isometric contraction than in resting muscle (Fig. 3), while the intensity of the 1,1 reflection is 100% ($\pm 25\%$, s.d. on nine muscles) stronger. Thus the intensity ratio $I_{1,1}/I_{1,0}$ in contracting muscle is about 1.6 compared with 0.4 in relaxed muscle at rest length^{1,10}. While it may be possible to explain the change in this ratio simply by azimuthal movement of the cross bridges, it is difficult to account for the large decrease in the absolute intensity of the 1,0 reflection by these means alone. For example, in Lymn's models, when the absolute change in the 1,0 reflection is calculated on going from his models IV or V (relaxed, long cross bridges) to model I (contracting) by comparing the intensity of these reflections with the 0,0 reflection, the 1,0 decreases by less than 10%, and does not account for the experimentally observed change: models with more than six cross bridges in projection would predict an even smaller change. Moreover, Lymn's models represent movement of all the cross bridges: there is evidence^{3,11} that suggests that not all of the cross bridges are attached to the thin filaments during contraction, and in this case, the changes predicted by Lymn's models IV and V would be even lower.

By rejecting Lymn's model we do not, of course, deny that there must be an azimuthal component to the cross-bridge movement; it is difficult to see how muscle could function efficiently without some azimuthal movement. On the evidence of our approximate calculations, however, it seems necessary to have a component of radial motion to account for the observed X-ray changes, and indeed such movement would also be necessary to allow the muscle to function at the different interfibrillar spacings found at different sarcomere lengths. Above all, we stress that it is

pointless to attempt to reach detailed conclusions about cross-bridge movement by modelling studies alone until all the main components of the unit cell have been characterised adequately.

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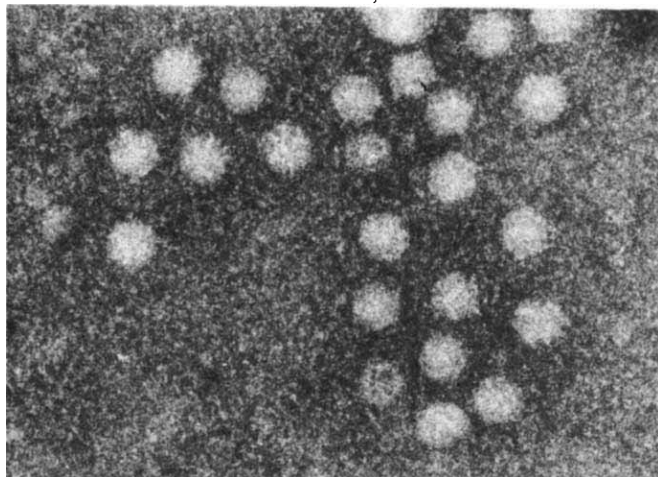
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Morphology of the GB hepatitis agent

ATTEMPTS to isolate hepatitis A virus have been in progress for several decades. An important advance was the finding that the marmoset served as a permissive animal host for this virus¹, but it became obvious that more than one agent was active. The pedigree of one agent was established as MS-1 (hepatitis A) isolate, while the other, which was demonstrably different by neutralisation and cross-challenge experiments, was designated GB. The GB infectious serum was obtained on the third day of jaundice from a surgeon in Chicago who developed a mild form of acute hepatitis. This serum induced hepatitis in all four marmosets inoculated. A pool of infectious GB serum marmoset (passage 11) was prepared from the total bleeding of nine white-lipped marmosets (*Saguinus* sp.) 19–24 d after inoculation and during the early acute phase of hepatitis as indicated by an increase of serum transaminase levels. This pool, designated H 205 GB pass 11, has an infectivity of $10^{4.5}$ marmoset ID₅₀. We have now examined the GB agent electron microscopically, and found that it is smaller and more fragile than the MS-1 strain², and therefore also the identical CR326 isolate³ of hepatitis A, which have the morphological appearances of recognised, small cubic viruses measuring about 27 nm in diameter.

Serum for examination was chosen from the period of maximum infectivity— $10^{4.9}$ ID₅₀—as established by further

Fig. 1 Aggregate of small spherical particles 20–22 nm in diameter found in the highest infectivity serum of marmoset infected with the GB agent. In this particular specimen the infectious marmoset serum had been reacted with recovery serum. $\times 275,000$.



passage in marmosets. Because immune electron microscopy has proved particularly useful in the investigation of viral hepatitis⁴, we examined the sera in the presence of convalescent serum from marmosets. Preinfection and normal marmoset sera were used as controls.

A prolonged search of the infectious serum reacted with convalescent serum revealed a few relatively small aggregates (immune complexes) of particles (Fig. 1), which were spherical and resembled parvoviruses. But aggregates were also found in infectious serum that had been reacted with normal serum. This implied that the infectious serum itself contained immune complexes. Subsequent examination of the serum itself confirmed that this was correct. Figure 2 illustrates an immune complex in GB-positive marmoset serum, revealing another distinctive feature of this virus. Several of the particles showed the rim staining associated with empty capsids and some of the empty capsids were fragmented further to reveal crescent-shaped forms.

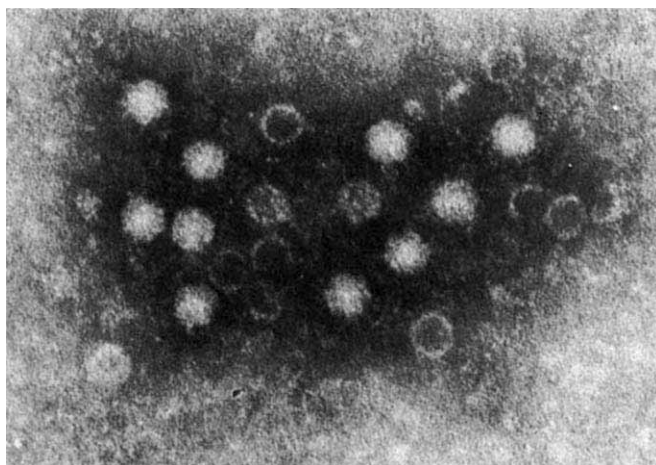


Fig. 2 Aggregate of 20–22-nm particles present in marmoset serum with no added antibody. This complex shows empty particles with ring staining and those even further degraded to crescentic forms. $\times 275,000$.

These findings are interesting from two points of view: morphology and immunology. Having recently examined the MS-1 hepatitis A isolate in chimpanzee faeces⁵, we have been able to establish that there is a considerable size difference between MS-1 and GB isolates. MS-1 has a peak size distribution between 28 and 30 nm, whereas the GB isolate has its peak size distribution between 20 and 22 nm. In addition, the GB agent seems to be more fragile, as the crescent-shaped forms seen in Fig. 2 were a recurrent feature, whereas they were not seen with MS-1. The morphological characteristics of GB are in accord with filtration data⁶ which suggested that the virus was about 20 nm in diameter. The second point of interest concerns the fact that the virus was present in the serum in the form of immune complexes. This finding too was in good agreement with published data. Hilleman⁷ demonstrated brief anticomplementary activity in early acute phase sera of hepatitis A illness and extrapolated that immune complexes might also form in the course of this infection.

The marmoset sera examined were near the limits of sensitivity of direct negative staining for electron microscopy. An infectivity titre of $10^{4.5}$ ID₅₀ per ml might be considered too low for visualisation of virus. It may have been the aggregates that made the virus visible. Similar structures were not seen in normal marmoset sera.

There is considerable interest, particularly in the United States, in the possibility that not all cases of viral hepatitis can be classified as either A or B. This has led to the suggestion of a third group, which could consist of several conditions and which at present is termed either human viral hepatitis type C⁸ or non-A:non-B hepatitis⁹. The GB agent that we describe here does not belong morphologically to either

hepatitis A or B and it may well be a third agent causing viral hepatitis in man.

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Immunological cross reactivity of Mason-Pfizer monkey virus with type C RNA viruses endogenous to primates

THE Mason-Pfizer monkey virus (MPMV) was originally isolated from a spontaneous mammary carcinoma of a rhesus monkey^{1,2}. Further isolates have been described from normal and malignant rhesus monkey tissues³. There have also been reports of viruses⁴⁻⁷, nucleic acids⁸ and antigens⁹ related to MPMV in human tissues. Viruses of the MPMV group have a density in sucrose of 1.16 g cm^{-3} (refs 10 and 11) and possess a segmented 60-70S RNA viral genome¹⁰⁻¹² which contains polyadenylate sequences of about 200 nucleotides¹³. In addition, MPMV contains a reverse transcriptase^{14,15} and structural polypeptides whose number and molecular weights resemble those of known type C RNA viruses¹⁶⁻¹⁷. In spite of these similarities with type C viruses, available evidence indicates that MPMV falls into a distinct group. This conclusion is based on morphological features that distinguish MPMV from type C viruses¹⁸, as well as differences in the biochemical properties of their reverse transcriptases¹⁵. Further, MPMV lacks detectable immunological cross reactivity with the 30,000 molecular weight (p30) major structural polypeptides of mammalian type C viruses^{5,16}. The antigenic determinants shared by p30s of known mammalian type C viruses have been used extensively in the identification of new virus isolates and in the analysis of immunological relationships between type C viruses^{19,20}. Other type C virus structural components such as gp70 also have been shown to possess broadly reactive antigenic determinants^{21,22}. We have now purified and characterised the gp70s of an endogenous virus of the baboon, an

old world primate and of MPMV. Our results provide evidence that MPMV is immunologically related to endogenous type C viruses of primates.

Viruses, isopycnicly banded in sucrose from tissue culture fluids, were provided by J. Gruber, Office of Resources and Logistics, Virus Cancer Program, NCI. Endogenous type C viruses of the *Papio cynocephalus* baboon²³ and of the cat, RD114²⁴, were used. Other type C viruses included Rauscher²⁵ and AKR-murine leukaemia virus (MuLV)²⁶, the Rickard strain of feline leukaemia virus (FeLV)²⁷, and infectious type C viruses of the woolly monkey²⁸ and gibbon ape²⁹. MPMV was grown in human or rhesus monkey cells; type C viruses were propagated as indicated below. Antisera, prepared by immunisation of goats with Tween-ether-disrupted viruses, and swine anti-goat immunoglobulin G were given by R. Wilsnack, through the Office of Resources and Logistics, NCI.

The gp70s of baboon type C virus, grown in canine thymus cells, and of MPMV, propagated in human cells, were isolated as described before²². In addition, the major structural polypeptides of molecular weight 30,000 (p30) and 26,000 (p26) of the baboon type C virus and MPMV, respectively, were isolated from the same virus preparations according to published methods²⁰. Each purified antigen was ¹²⁵I-labelled by the chloramine T method of Greenwood *et al.*³⁰ and shown by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate³¹ to be at least 90% radiochemically pure.

Antisera prepared against various mammalian type C viruses and MPMV were first compared for their abilities to bind the gp70 and p30 structural polypeptides of the baboon virus and MPMV. As Table 1 shows, ¹²⁵I-labelled gp70 of the baboon virus was

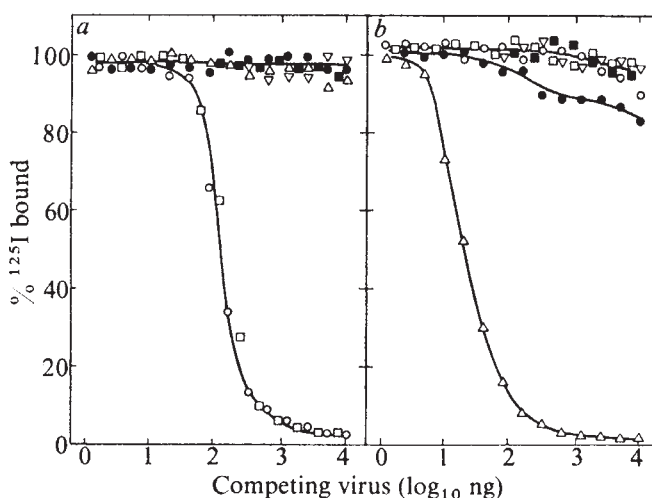


Fig. 1 Comparison of the immunological reactivities of MPMV and representative type C virus isolates of different mammalian species in homologous immunoassays for the 70,000 molecular weight glycoproteins of MPMV and of the endogenous *Papio cynocephalus* baboon type C virus. Unlabelled detergent-disrupted²⁰ viral antigens were tested at serial twofold dilutions for their ability to compete with ¹²⁵I-labelled viral gp70 for binding limiting amounts of antiserum. Reaction mixtures contained 0.01 M Tris-HCl, pH 7.8, 0.1 M NaCl, 0.001 M EDTA, 0.1% Triton X-100 and 1% bovine serum albumin in a volume of 0.15 ml. After incubation of antiserum and unlabelled competing viral antigen at 37 °C for 1 h, the appropriate ¹²⁵I-labelled viral gp70 was added in 0.05 ml and incubation continued for 3 h at 37 °C and a further 18 h at 4 °C. Undiluted swine anti-goat immunoglobulin G (0.025 ml) was then added to each tube and reaction mixtures were incubated for 3 h at 4 °C, centrifuged at 2,500 r.p.m. for 15 min, and the resulting precipitate was analysed for radioactivity. The viruses tested included MPMV propagated in either human (○), or rhesus monkey cells (□), and type C viruses including baboon (△), RD114 (●), gibbon ape (▲) and woolly monkey viruses (■), all grown in human cells and Rauscher MuLV grown in mouse cells (▽). Viral protein concentrations were determined by the method of Lowry *et al.*³⁶. *a*, Homologous MPMV immunoassay in which limiting antiserum against MPMV was used with ¹²⁵I-labelled MPMV gp70; *b*, homologous baboon type C virus gp70 immunoassay in which limiting antiserum against baboon virus was used with ¹²⁵I-labelled baboon viral gp70.

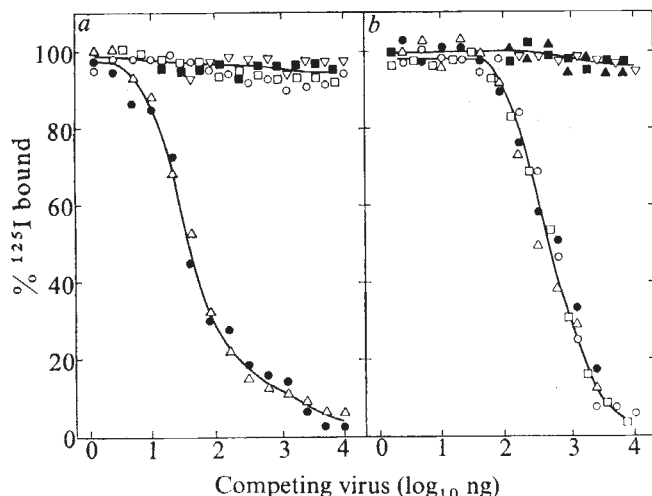


Fig. 2 Interspecies competition immunoassays for the 70,000 molecular weight glycoproteins of MPMV and the baboon type C virus. The viruses tested and symbols used for each are indicated in the legend in Fig. 1. Immunoassays were performed as described in Fig. 1 and included: *a*, heterologous immunoassay in which limiting antiserum to RD114 was used with 125 I-labelled baboon virus gp70; and *b*, heterologous immunoassay in which limiting antiserum to MPMV was used with 125 I-labelled baboon type C virus gp70.

precipitated at titres of 1:10,000 and 1:150,000, respectively, by antisera to the endogenous baboon virus and to a closely related endogenous virus of the cat, RD114. The maximum extent of precipitation was about 90% with each antiserum. These results indicated preservation, during purification, of antigenic determinants of the baboon viral gp70. The precipitation titres of the same antisera against baboon viral p30 were 10-20-fold greater, consistent with previous observations that antisera to type C viruses prepared in heterologous hosts exhibit higher titres against p30 than gp70 (ref. 21). Antisera to several other mammalian type C viruses also precipitated baboon viral p30 at titres ranging from 1:2,900 with anti-AKR-MuLV to 1:18,000 with antiserum to FeLV. None of these antisera, however, showed detectable reactivity against baboon viral gp70 (titres less than 1:10).

As Table 1 shows, two separately prepared antisera to MPMV each demonstrated a high level of reactivity against 125 I-labelled MPMV gp70. The maximum extent of precipitation was around 70%, and titres for 10% binding were 1:450,000 and 1:280,000, respectively. The immunoprecipitation titres of these same antisera against the major structural polypeptide of MPMV were even higher. It is interesting (Table 1) that both antisera also demonstrated significant binding of baboon viral gp70. The precipitation titres were 1:640 and 1:400, with maximum precipitation

of 69% and 53% of the labelled baboon viral gp70 probe. The anti-MPMV sera also precipitated 125 I-labelled RD114gp 70 at titres of 1:1,000 and 1:800, but showed no detectable reactivity against the gp70s of FeLV, Rauscher-MuLV or woolly monkey virus (data not shown). These results suggested that MPMV shares antigenic determinants with gp70s of viruses of the baboon-RD114 virus group but not with gp70s of other mammalian type C viruses. Findings that the same MPMV antisera failed to precipitate p30s of either the baboon virus (Table 1) or RD114 (data not shown) are consistent with previous reports^{5,16}, indicating lack of relatedness of the major structural polypeptide of MPMV with analogous polypeptides of mammalian type C viruses.

To characterise the gp70 antigenic determinants cross reactive of competition immunoassays were developed. As Fig. 1*a* shows, in an immunoassay utilising anti-MPMV serum to precipitate 125 I-labelled MPMV gp70, MPMV, propagated in either human or rhesus monkey cells, competed with high efficiency. In contrast, several type C viruses tested, including baboon and RD114, showed no detectable reactivity. In an immunoassay utilising antiserum to the baboon virus to precipitate 125 I-labelled baboon viral gp70 (Fig. 1*b*), only the homologous virus competed efficiently. RD114 competed 10-15% of the 125 I-labelled gp70 at the highest virus concentration tested, while none of the other mammalian type C viruses or MPMV showed any reactivity. Thus, MPMV and baboon viral gp70s could be distinguished readily in these two assays.

Immunoassays designed to detect more broadly reactive antigenic determinants of the baboon viral gp70 utilised antisera against RD114 or MPMV to precipitate 125 I-baboon viral gp70. Reciprocal studies with 125 I-MPMV gp70 were not possible because available antisera to baboon-RD114 viruses failed to precipitate this polypeptide. As Fig. 2*a* shows, baboon and RD114 viruses competed with similar high efficiency in the heterologous anti-RD114: 125 I-baboon viral gp70 immunoassay, whereas MPMV and the other type C viruses tested were all unreactive. In the anti-MPMV: 125 I-baboon viral gp70 assay (Fig. 2*b*), MPMV, baboon and RD114 viruses each competed with similar efficiency, although none of the other type C viruses, including several grown in human or rhesus monkey cells, showed any detectable reactivity. Purified MPMV and baboon viral gp70s were found to possess reactivities identical to those of their respective parental viruses in each of the competition immunoassays used in the studies reported here (data not shown). These findings indicate that the shared antigenic determinants of MPMV and the baboon type C virus were associated with 70,000 molecular weight polypeptides of each virus.

Two lines of evidence exclude the possibility that the immunological cross reactivity of MPMV with baboon and RD114

Table 1 Comparison of abilities of antisera to bind 125 I-labelled MPMV and baboon virus polypeptides

Antisera to	Cells propagated in	Antisera titres for binding 125 I-labelled polypeptide of*							
		baboon virus gp70		baboon virus p30		MPMV gp70		MPMV p30	
		titre	maximum precipitation (%)	titre	maximum precipitation (%)	titre	maximum precipitation (%)	titre	maximum precipitation (%)
Baboon virus	Human	10,000	90	200,000	88	<10	<5	<10	<5
RD114	Human	150,000	89	1,200,000	90	<10	<5	<10	<5
Gibbon ape virus	Human	<10	<5	5,000	70	<10	<5	<10	<5
Woolly monkey virus	Human	<10	<5	12,000	85	<10	<5	<10	<5
FeLV	Cat	<10	<5	18,000	67	<10	<5	<10	<5
Rauscher MuLV	Mouse	<10	<5	4,100	75	<10	<5	<10	<5
AKR-MuLV	Mouse	<10	<5	2,900	80	<10	<5	<10	<5
MPMV	Rhesus monkey	640	69	<10	<5	450,000	70	1,600,000	60
MPMV	Human	400	53	<10	<5	280,000	68	1,700,000	61

* 125 I-labelled viral polypeptides (10,000 c.p.m.) were incubated with antisera at serial twofold dilutions in reaction mixtures containing 0.01 M Tris-HCl, pH 7.8, 0.1 M NaCl, 0.001 M EDTA, 0.1% Triton X-100 and 1% bovine serum albumin, in 0.2 ml. After 3 h incubation at 37°C and a further 18 h at 4°C reaction mixtures were diluted to 1.0 ml, mixed with 0.1 ml pig anti-goat immunoglobulin G, and incubated for 3 h at 4°C. The immunoprecipitates were centrifuged at 2,500 r.p.m. for 15 min and analysed for radioactivity. Antiserum titre is expressed as reciprocal of the highest serum dilution able to bind 10% of the appropriate 125 I-labelled viral polypeptide. Maximum precipitation values represent the percentage of 125 I-labelled antigen bound at the highest antiserum concentration tested.

viruses was due to contamination of MPMV stocks with a known type C virus. First, the patterns of reactivity of each virus in the various competition immunoassays for gp70 used in these studies distinguished the type C viral cross-reactive determinants of MPMV from those of baboon or RD114 viruses or any of several other type C viruses tested. Furthermore, the MPMV preparations used here did not cross react in immunoassays^{5,16} which sensitively detect the major structural polypeptides of all known mammalian type C viruses (data not shown).

The results thus indicate that there must either be antigenic relatedness of virus-coded polypeptides of MPMV and type C viruses of the baboon-RD114 group or that these viruses incorporate some cross-reactive cellular protein. The preservation of immunological characteristics of a virion polypeptide after virus growth in cells of very different species is generally considered strong evidence that the polypeptide is virus coded. This criterion could be met readily for baboon and RD114 viruses. These viruses have a wide host range, and the antigenic specificities of their gp70s were stable when they were grown in cells of diverse hosts. Since MPMV is only infectious for cells of primate origin, this criterion cannot as adequately be satisfied for MPMV gp70. But the absence of the cross-reactive antigen in other viruses grown in human or rhesus monkey cells excludes the nonspecific incorporation of this antigen by MPMV. Moreover, our results indicate that purified MPMV gp70 displayed the same antigenic specificities as the intact virus. Thus, if the type C viral cross-reactive antigen associated with MPMV is cellular rather than MPMV coded, it must specifically be associated with MPMV and possess physical properties very similar to those of MPMV gp70.

The more likely alternative is that MPMV gp70 has both MPMV-specific antigenic determinants as well as others that are shared with type C viruses of the baboon-RD114 group. As previous findings have indicated, the reverse transcriptase¹⁵ and major structural antigen^{5,16} of MPMV lack detectable cross-reactivity with analogous components of known mammalian type C viruses. The rhesus monkey, a natural host of MPMV, contains genetic information for an endogenous type C virus related to the baboon type C virus³² and expresses antigens of this virus in its normal tissues³³. Moreover, experimental evidence has established that recombination can occur between infectious type C viruses and endogenous type C viruses of the cells in which they are propagated^{34,35}. Thus it can be speculated that MPMV may have initially acquired its gp70 by a mechanism involving recombination with an endogenous primate type C virus.

Like MPMV, several reverse transcriptase-containing viruses, including primate syncytial viruses, visna virus and mouse mammary tumour virus, have morphological characteristics that differ from those of type C viruses. So far studies have failed to establish an immunological relationship of any of these viruses with mammalian type C viruses in tests of either their reverse transcriptases or major structural polypeptides. The application of radioimmunological techniques for detection of other viral components should help to determine whether any of these viruses like MPMV, is immunologically, and thus possibly evolutionarily, related to type C RNA viruses.

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Newcastle disease virus mRNA lacks 2'-O-methylated nucleotides

MESSENGER RNA molecules from various sources contain methylated nucleotides at the 5' terminus of the RNA molecules¹⁻⁹. The structure at the 5' terminus of most cellular and viral mRNAs consists of a terminal base-methylated nucleotide linked to a ribose-methylated nucleotide through a 5' to 5' pyrophosphate bond, such as m⁷G(5')ppp(5')AmpNp or m⁷(5')ppp(5')GmpNp. Initiation and subsequent *in vitro* translation of mRNA are dependent on the presence of m⁷G in a blocked 5' structure^{10,11}. Thus, methylation (m⁷G) seems to serve an important function in the translation (and/or control) of viral and cellular mRNA. The role of 2'-O-methylation has not been identified. The widespread presence of 2'-O-methylation in mRNA from diverse systems suggested that they might be a common feature of all eukaryotic mRNAs⁴⁻⁹. We report here that the 5'-terminal structure of Newcastle disease virus (NDV) mRNA synthesised *in vitro* is m⁷G(5')ppp(5')GpPyp. The structure isolated from NDV mRNA differs from those reported for all other mRNAs synthesised *in vitro*⁵⁻⁸ in that the RNA lacks 2'-O-methylated nucleotides. During the preparation of our report we learned that the 40S RNA genome of Sindbis virus¹⁴ and the 26S RNA synthesised in Sindbis virus-infected cells¹⁵ have the structure m⁷G(5')ppp(5')Gp. In addition tobacco mosaic virus has m⁷G(5')ppp(5')Gp at the 5' end of the viral genome^{12,13}.

Newcastle disease virus (NDV), and avian paramyxovirus contain a virion-associated RNA polymerase¹⁶ that transcribes the 50S RNA genome *in vitro* into 18S molecules. These molecules are complementary in base sequence to the genome and contain polyadenylic acid sequences¹⁷. We^{18,19} have demonstrated the presence of a methyltransferase activity in purified NDV which catalyses the incorporation of the methyl group from ³H-S-adenosyl-L-methyl methionine (SAM) into the messenger RNA synthesised *in vitro* by the virion-associated transcriptase. The procedures for growth and purification of NDV, the reaction mixture for *in vitro* RNA synthesis in the presence of SAM, the methods for isolation and purification of the *in vitro* RNA product, and the DEAE-cellulose chromatography for analysis of oligonucleotides have been described before¹⁸.

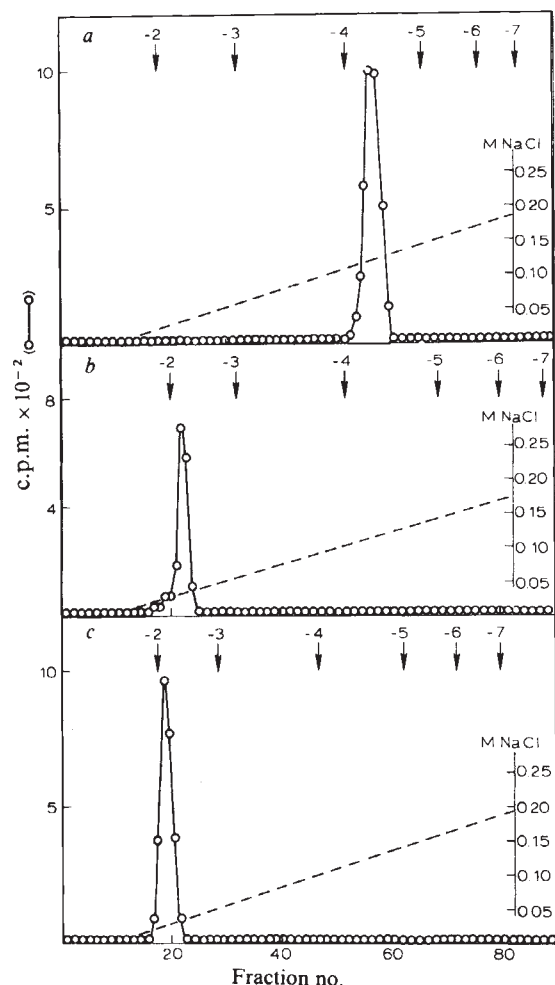


Fig. 1 DEAE-cellulose chromatography of ^3H -methyl-labelled RNA products after enzymatic digestions. *a*, Purified ^3H -methyl-labelled RNA was denatured in a boiling water bath for 3 min and a 0.5-ml sample in 10 mM Na acetate, pH 4.5, was incubated with 4 U of RNase T_2 (Calbiochem) at 37 °C for 5 h. The digest was chromatographed on a 0.6 $\times$ 17-cm column of DEAE-cellulose with 3 mg of RNase A-digested rat liver ribosomal RNA as an oligonucleotide marker and a portion of each fraction was analysed for radioactivity as described before¹⁸. The salt gradient is indicated by the dotted line. The elution positions of marker oligonucleotides are indicated by arrows and expressed as their net negative charges. The fractions containing radioactivity were desalted by dialysis against water, lyophilised, resuspended in buffer, and a portion of the RNase T_2 -resistant oligonucleotide was digested with either alkaline phosphatase or penicillium nuclease before rechromatography. *b*, Alkaline phosphatase digest of (*a*). A sample (0.5 ml) of the oligonucleotides obtained from the radioactive region shown in (*a*) was incubated in 20 mM Tris-HCl, pH 8.0, with 2.5 mg (125 U) of bacterial alkaline phosphatase (Worthington) for 1 h at 37 °C. The sample was then chromatographed on DEAE-cellulose as in (*a*). *c*, Penicillium nuclease digest of (*a*). A sample (0.5 ml) of the oligonucleotides obtained from the radioactive region shown in (*a*) was incubated in 10 mM Na acetate, pH 6.0, with 100 μg of penicillium nuclease (Yamasa Shoyu Co.) for 1 h at 37 °C. The sample was then chromatographed on DEAE-cellulose as in (*a*).

To determine the specific site of methylation, ^3H -methyl-labelled RNA was synthesised in the presence of SAM and then digested with RNase T_2 (which degrades RNA to nucleoside-3'-monophosphates in the absence of 2'-O-methyl groups), and the resulting products were analysed on a DEAE-cellulose column. A single peak (Fig. 1*a*) of ^3H radioactivity eluted between the -4 and -5 charged markers identical to the position previously reported¹⁸ for the ^3H -methyl-labelled RNA after KOH hydrolysis. Structures which should elute in the position described include

pNmpNp, mNpppNp or NppNmpNp. The labelled material was desalted and a portion was treated with bacterial alkaline phosphatase (which removes terminal monoesterified phosphate groups) and reanalysed on DEAE-cellulose. A single peak of radioactivity now eluted between the -2 and

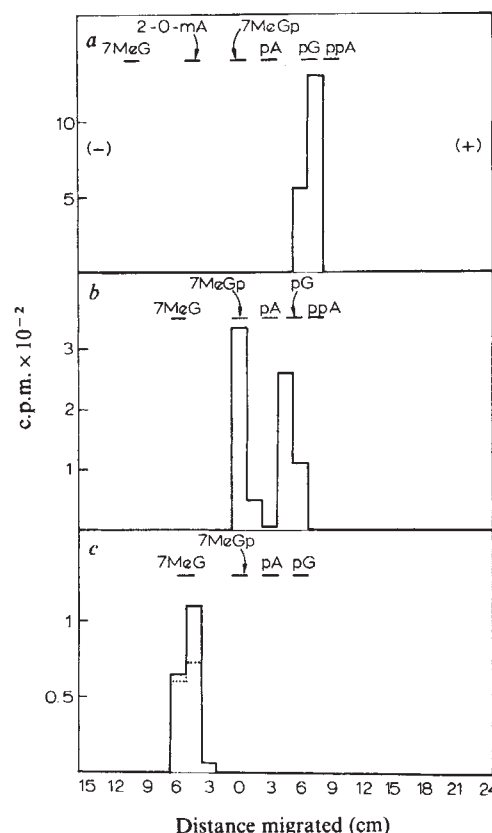


Fig. 2 Paper electrophoresis of the terminal oligonucleotide. *a*, Purified ^3H -methyl-labelled RNA was digested with penicillium nuclease as described in Fig. 1*c* and then examined by high voltage paper electrophoresis using a procedure similar to that of Abraham *et al.*⁸. Unlabelled markers (20 μg in 2 μl) and labelled samples (2–10 μl) were spotted on Whatman 3 MM paper (33 $\times$ 39 cm) and separated by electrophoresis in pyridine: acetic acid: water (1:10:89), pH 3.5, at 1,200 V for 2 h, using a Brinkmann MINI 68 high voltage cold plate electrophoresis apparatus. The paper was then dried and the markers located under ultra-violet light. The paper was cut into pieces (1.5 cm $\times$ 1.5 cm) and the radioactivity in each piece was counted in 10 ml of toluene containing PPO and POPOP. *b*, The ^3H -labelled material migrating as pG (*a*) was recovered from the paper as described below, digested with nucleotide pyrophosphatase as follows and then subjected to high voltage paper electrophoresis. Paper squares which contained radioactivity were removed from the counting vials, washed twice in toluene to remove fluors and dried. One side of the square was cut to form a point and water was allowed to ascend up to the point. The square was wrapped in aluminium foil (point sticking out) and suspended point down in a test tube so that the square was 1 cm from the bottom of the tube. Labelled samples were then recovered by centrifuging in an International PR-2 centrifuge (1 min, 1,000 r.p.m.). The labelled sample was digested with 0.025 U of nucleotide pyrophosphatase in 0.5 ml 20 mM Tris-HCl, pH 7.5, 1 mM MgCl_2 for 15 min at 37 °C. The sample was lyophilised, resuspended in 10 μl of water and spotted on Whatman 3 MM paper. Electrophoresis and assay were performed as in (*a*). *c*, The labelled materials migrating as m⁷Gp and pG (*b*) were recovered from the paper, digested with alkaline phosphatase, and then analysed by high voltage paper electrophoresis. The radioactivity was recovered from the paper squares as described in (*b*), and alkaline phosphatase digestion was performed as described in Fig. 1*b*. The samples were lyophilised, resuspended in 10 μl of water and spotted on Whatman 3 MM paper. Electrophoresis and assay of the materials which had migrated as m⁷Gp(–) and pG(–) were performed as in (*a*).

Table 1 Periodate oxidation and removal of the blocking base by aniline

RNA Sample	C.P.M. Treated	C.P.M. (%) Recovered in ethanol*	C.P.M. (%) Recovered in precipitated RNA
¹⁴ C-UMP-labelled 18+28S rRNA	9,700	824 (8.6)	8,795 (91.4)
³ H-methyl-labelled product RNA	4,400	4,029 (92.1)	347 (7.9)

*Values in this column represent the total c.p.m. recovered from four ethanol precipitations.

—3 charged markers (Fig. 1b), indicating the removal of a single phosphate group. This result eliminates structures such as pNmpNp because two phosphate groups would have been removed by alkaline phosphatase. To distinguish between structures such as mNpppNp and NppNmpNp, a portion of the methyl-labelled oligonucleotide eluting in Fig. 1a was treated with penicillium nuclease (which degrades RNA to nucleoside-5'-monophosphates, regardless of the presence of any 2'-O-methyl groups, and also contains a 3'-phosphatase activity), and analysed by DEAE-cellulose chromatography. The elution pattern (Fig. 1c) did not differ from the pattern obtained after RNase T₂ digestion and alkaline phosphatase treatment (Fig. 1b), suggesting the absence of any 2'-O-methyl groups.

The ³H-methyl-labelled structure at the 5' terminus of NDV mRNA was analysed further by high voltage paper electrophoresis. After treatment with penicillium nuclease, the resulting material migrated on paper near the pG marker (Fig. 2a). This material was eluted from paper,

treated with nucleotide pyrophosphatase (which cleaves pyrophosphate linkages), and reanalysed by paper electrophoresis. Treatment with nucleotide pyrophosphatase released two equally labelled products (Fig. 2b), one of which comigrated with the pG marker. These two products most likely represent m⁷Gp and m⁷Gpp, respectively. Both products were recovered, treated with alkaline phosphatase, and reanalysed by paper electrophoresis. Treatment with alkaline phosphatase removed the remaining phosphate groups, and ³H-methyl radioactivity of both products migrated with authentic m⁷G (Fig. 2c). These results show conclusively that the ³H-methyl label resides solely in m⁷G. The fact that two products were obtained after nucleotide pyrophosphatase digestion (Fig. 2b) proves that the 5' terminus contains three phosphates. Cleavage of a diphosphate would have given a single product.

Thus the methylation of NDV complementary RNA exists as a m⁷G and can be depicted as m⁷GpppNp, Npppm⁷Gp or m⁷Gpppm⁷Gp. If a 5'-5' linkage exists between the two nucleosides, the blocking nucleoside (m⁷G or N) will contain free 2'- and 3'-hydroxyl groups and will be subject to periodate oxidation and removal by treatment with aniline⁸. When ³H-methyl-labelled RNA was oxidised with potassium periodate, followed by treatment with aniline, less than 8% of the methyl radioactive label was recovered with ethanol-precipitated RNA (Table 1), indicating that the m⁷G resides as a blocking base. As a control for experimental manipulations, parallel treatment of ¹⁴C-UMP-labelled 18 and 28S ribosomal RNA resulted in more than 91% recovery of the radioactive label in precipitated RNA (Table 1). These results indicate that the blocking base is m⁷G. The fact that almost all the radioactivity was removed eliminates m⁷Gpppm⁷G, because only 50% of the radioactivity would have been removed from the latter structure. Thus the 5'-terminal structure must be m⁷G(5')pppNp. Since more than 90% of the methyl label in NDV mRNA is released as m⁷G by periodate and aniline treatment, and since the data in Fig. 1 suggest the absence of any 2'-O-methyl groups, the level of any additional methylation, such as internal base methylations (N⁶-methyladenosine²⁻⁴ or 5-methyl cytosine¹³), cannot exceed 8% (the amount of methyl label product which was not released by periodate and aniline treatment, Table 1).

To identify the unknown nucleotide in the methylated 5'-terminal structure, ³H-methyl-labelled RNA was treated with either RNase A or RNase T₁ and analysed on DEAE-cellulose columns. Treatment with RNase T₁ alone (which cleaves on the 3' side of guanylic acid residues) resulted in a methylated, RNase-resistant structure which eluted between the -4 and -5 negatively charged markers (Fig. 3a). Since the elution position is identical to that obtained by base hydrolysis¹⁸ and ribonuclease T₂ treatment (Fig. 1a), the second base in the capped structure must be G. RNA treated in a similar manner with RNase A (specific for the 3' side of pyrimidine nucleoside linkages) eluted between the -5 and -6 negatively charged markers (Fig. 3b). Thus the 5' terminus of NDV RNA seems to be m⁷G(5')ppp(5')GpPyp.

Since NDV mRNA does not contain any 2'-O-methylated nucleotides, it should serve as a valuable control in experiments designed to elucidate the role of 2'-O-methylated nucleotides in other eukaryotic mRNAs.

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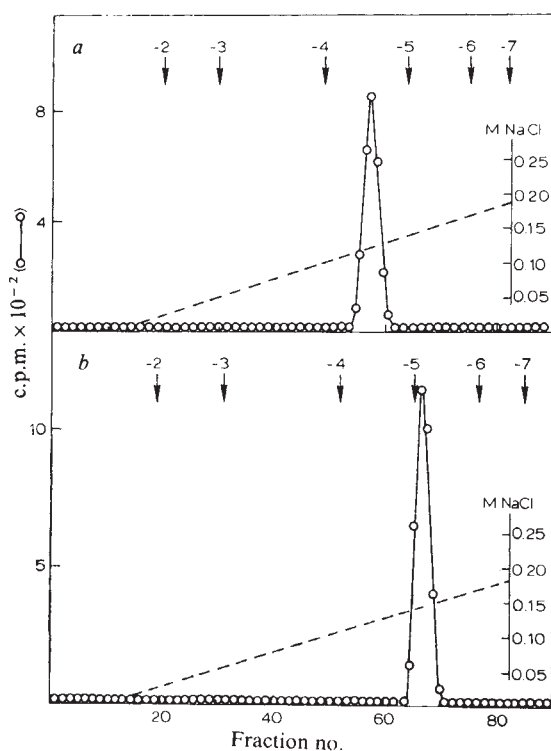


Fig. 3 DEAE-cellulose chromatography of ³H-methyl-labelled RNA after RNase digestion. Purified ³H-methyl-labelled RNA was digested with RNase T₁ (a) or RNase A (b) and chromatographed on DEAE-cellulose as described in Fig. 1. Labelled samples were resuspended in 0.5 ml of 5 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.3 M NaCl and incubated with 2 μg (678 U ml⁻¹) RNase T₁, or with RNase A (50 μg ml⁻¹) at 37 °C for 30 min. The salt gradient is indicated by the dotted line. The elution positions of marker oligonucleotides are indicated by arrows and expressed as their net negative charges.

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DNA endoreduplication and polyteny understood as evolutionary strategies

DNA endoreduplication and related phenomena (such as endomitosis, polyteny, nuclear restitution and somatic polyploidy in general) are widespread over the animal and plant kingdoms, although they occur most frequently among insects and angiosperms¹⁻³. The systematic restriction to certain phyla and species has been interpreted in terms of high genetic control of such events³, whereas the characteristic developmental pattern of various degrees of endopolyploidy has been considered as an expression of their functional role in differentiation and synthesising capacity of the cells^{2,4,5}. Recently,

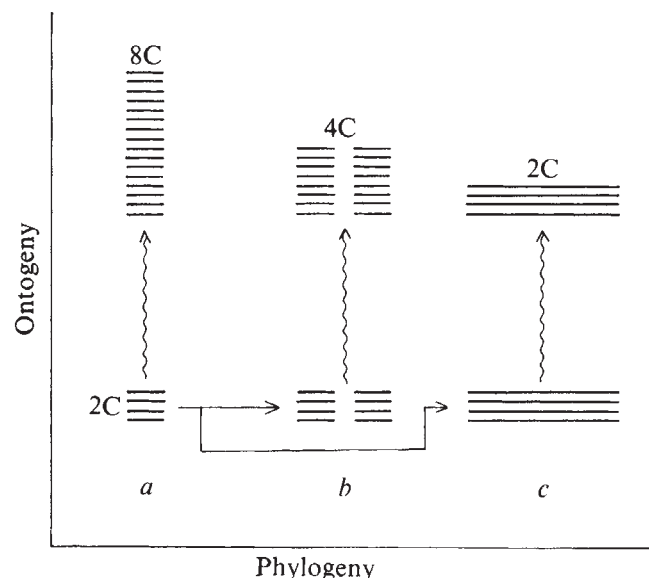


Fig. 1 Diagram to illustrate the relationship between phylogenetic increase in basic (2C) nuclear DNA and ontogenetic increase by somatic polyploidy. The scheme assumes a diploid species with 4 chromosomes and low DNA content (a), a generative-tetraploid species with twice as many chromosomes and DNA (b), and a diploid species with 4 enlarged chromosomes with 4 times the DNA content of species a (c). During differentiation the most active cells need a certain DNA mass which is essentially similar in all species of comparable complexity. To achieve this amount of DNA, 4 endocycles must occur in species a, two in species b, but none in species c. The scheme also suggests that phylogenetic DNA increase occurs tandemly, while ontogenetic increase occurs laterally. Polyteny is, therefore, the automatic consequence of DNA endoreduplication, if the sister chromosomes are not secondarily separated from each other, for example, by endomitotic chromosome coiling. Note that species b may, in addition to generative polyploidy, develop 8C nuclei during ontogeny. Moreover, the trend to endopolyploidy may also increase, if the 2C DNA content is reduced during phylogeny (for example in the evolution of species).

however, any role of endoreduplication in cell differentiation has been questioned because of the existence of species apparently lacking endopolyploidy⁶. All previous discussions on endoreduplication, endopolyploidy and polyteny have, however, ignored the basic DNA contents of the species studied. We here report a relationship between the basic nuclear DNA content and the occurrence and degree of endopolyploidy. This strongly suggests that DNA endoreduplication can be regarded as an evolutionary alternative to the high nuclear DNA content that has been achieved in other species mainly by 'saltatory replications'.

As well as reviewing the huge literature on endopolyploidy, we have examined several species for the first time, using a Leitz MPV-2 scanning photometer interfaced to a PDP-8 computer (Digital Equipment). The following relationships could be detected. The lower the basic (2C) nuclear DNA content of a given species, the higher is the probability that endopolyploid or polytenic nuclei are developed in certain cells (Table 1). This negative relationship between 2C DNA content and maximum ploidy is surprisingly clear in relation to the rather small sample of animals and plants for which both data are available. Moreover, the same relationship is found among higher taxonomic groups (Table 2): Ciliata and Diptera, which have only low basic nuclear DNA contents, show a high tendency for endopolyploidisation and polytenisation; mammals and dicotyledonous plants, with intermediate amounts of DNA also develop, on an average, more intermediate levels of endopolyploidy; and Amphibia and monocots that possess the highest nuclear DNA contents, fail to show—at least higher—polyploidy levels.

Regarding the tissue-specific pattern of endopolyploidy and polyteny, the cells which display the highest levels are those which have been assumed to be the most active and specialised, such as salivary glands of insect larvae, the trophoblasts of mammalian embryos, the trophocytes of insect ovarioles, the suspensors of angiospermal embryos, and so on⁸. In this context it is worth mentioning that cases of differential DNA

Table 1 Nuclear DNA contents and maximum endopolyploidy levels in various plants and animals

Species	DNA (10^{-12} g per 2C nucleus)	Endopolyploidy (maximum C level)
<i>Capsella bursa-pastoris</i>	1.7	1,024*
<i>Cucurbita pepo</i>	2.6	384
<i>Phaseolus coccineus</i>	2.7	8,192
<i>Phaseolus vulgaris</i>	2.7*	2,048
<i>Tropaeolum majus</i>	2.7*	2,048*
<i>Helianthus annuus</i>	6.1-9.9*	16*
<i>Hordeum vulgare</i>	13.4	256
<i>Lathyrus ssp.</i>	9-20	64
<i>Vicia faba</i>	23.9-28.7	16
<i>Allium cepa</i>	35.5	32
<i>Tradescantia virginiana</i>	54.0	8
<i>Pinus sylvestris</i>	93.8	—
<i>Picea alba</i>	100.0	—
<i>Pinus resinosa</i>	138.0	—
<i>Drosophila melanogaster</i>	0.36	2,048
<i>Chironomus tentans</i>	0.5	32,768
<i>Bombyx mori</i>	1.0	524,288
<i>Aplysia californica</i>	2.0	200,000
<i>Rattus rattus</i>	6.1	4,096
<i>Gryllus domesticus</i>	12.0	16*
<i>Triturus alpestris</i>	58.0	—*
<i>Salamander salamander</i>	64.0	—*
<i>Ambystoma mexicanum</i>	76.0	—*

*DNA contents not taken from the literature but calculated from the data obtained by scanning densitometry of Feulgen-stained nuclei, using *Allium cepa* as a standard (2C = 35.5 pg (ref. 20)) to convert the relative values into pg. The other data have been taken from various reviews^{8,18,21,22}. Negative results on endopolyploidy do not exclude the possibility that some specialised cells may, nevertheless, develop polyploid nuclei, which have not yet been detected. With a few exceptions, however, the data strongly indicate a negative relationship between basic (2C) nuclear DNA content and the maximum endopolyploidy reached within a species.

Table 2 Range of nuclear DNA contents and endopolyploidy levels in various vegetal and animal taxa (arranged in systematic order)

Taxon	Range of 2C DNA content (pg) ²¹⁻²⁴	Range of endopolyploidy ⁸	Organ with the highest level ⁸
Bryophyta	1.5-9.0	4-16	Paraphyses
Pteridophyta	30.0-300.0	—	—
Gymnosperms	8.0-200.0	—	—
Angiosperms	0.6-200.0	4-24,000	Suspensor, endosperm
Mollusca	1.0-10.0	4-200,000	Giant neurones
Insecta	0.3-15.0	4-500,000	Salivary glands, Malpighian tubules
Pisces	0.9-284.0	—	—
Amphibia	6.3-168.0	—	—
Mammalia	5.0-7.0	4-4,096	Trophoblast

The table shows that taxa with high average nuclear DNA contents, unlike those with rather low DNA amounts, do not show endopolyploidisation. The degrees of polyploidy reached in a species are, with a few exceptions, negatively correlated to its basic nuclear DNA content (Table 1). This explains the high variation of ploidy levels seen in some taxa.

replication can be regarded as an economical and/or necessary variation of endoreduplication: DNA underreplication occurs in cells which no longer need the centromeric heterochromatin, probably because they will never divide again^{8,9}. DNA amplification has been envisaged as a local, transitory polytenisation in germ line cells that must not undergo endopolyploidisation¹⁰.

The following conclusions can be drawn from these findings. The differentiation and specialised function of certain cells of eukaryotic organisms evidently need a defined mass of DNA (and probably not only of protein-coding and ribosomal DNA) to maintain their regulatory and functional state. The necessary DNA content has been achieved in many species through two different ways: one is characterised by tandem duplications of DNA sequences (for example, by saltatory replications), leading to the increase of predominantly non-informative, repetitive DNA sequence families^{7,11,12}. The other way is by generative (or germ-line) polyploidy¹³, which yields, in addition, the advantage of possible gene dose effects in the 'laterally' multiplicated genome¹⁴⁻¹⁷, as well as the possibility to overcome the general limit of DNA increase. A third way may be seen in the tandem arrangement of polyploid chromosome sets, leading to larger chromosomes in a stable number (cryptopolyploidy¹⁸ which, however, is still a rather speculative idea).

When the phylogeny of an order, family or genus has not been accompanied by an increase in the basic nuclear DNA, however, the necessary DNA content is achieved during ontogeny, by various species- and tissue-specific degrees of somatic polyploidy. With regard to differentiated cells, this may result in a similar effect (Fig. 1). Thus, DNA endoreduplication, polyteny, and somatic polyploidy in general, evidently represent an evolutionary strategy which substitutes for a lack of phylogenetic increase in nuclear DNA. In spite of the evidence given for this interpretation in Tables 1 and 2 it should be generalised only with caution: it is a general feature of evolution that different strategies may be involved in the creation of even closely related species¹⁹.

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Internal inversion used to study regulation of the *int* gene in bacteriophage λ

Two events are essential for successful lysogenisation by bacteriophage λ : establishment of repression and integration of the viral genome into the bacterial chromosome. Integration is catalysed by the phage *Int* function¹. The *int* gene is situated at the distal end of the early leftward N

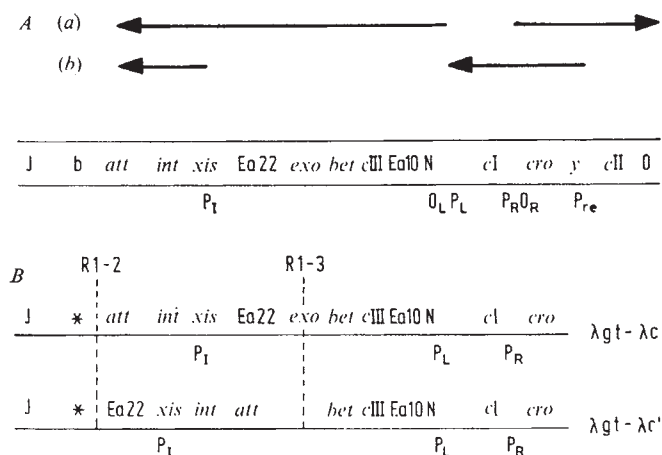


Fig. 1 Portion of the genetic map of bacteriophage λ showing transcription pattern and the structure of the reconstructed phages λ gt- λ C and λ gt- λ C'. A, After infection, transcription of the two early λ operons is initiated at P_L and P_R (arrows in (a)). In the establishment of lysogeny, transcription of the repressive gene originates at P_{re} and of the *int* gene probably at P_L . The synthesis of both transcripts (arrows in (b)) is activated by the positive regulators *cI* and *cIII*. B, *EcoRI* restriction sites and the genetic structure of the inversion has been determined before⁵. The stars denote the position of the *b* region of bacteriophage λ which was partly deleted during the construction of these phages. The genetic location of the genes responsible for the synthesis of the various protein bands was determined by the use of various known deletion⁷ and amber mutations (in my work, in preparation), and is consistent with previous reports⁸.

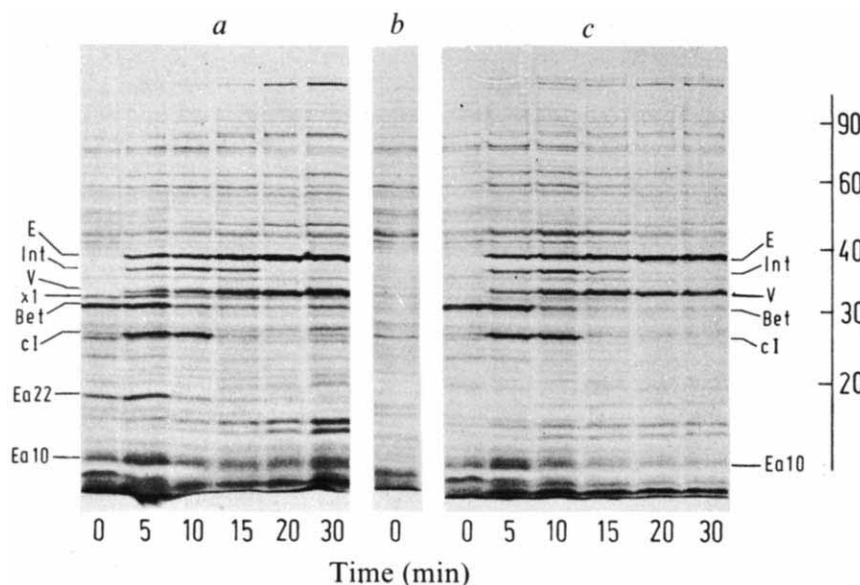


Fig. 2 Analysis of phage-directed protein synthesis. Cells were grown in HFCM medium⁸, irradiated with 1.6×10^4 erg mm⁻² and starved for 15 min at 37 °C before being infected at a multiplicity of 10. The infected cells were incubated in HFLA minimal medium⁸ and pulsed with ¹⁴C-mixed amino acids ($5 \mu\text{Ci min}^{-1}$) for 5-min intervals starting at the time designated. Samples were subjected to electrophoresis on SDS-polyacrylamide slab gels^{9,10} containing 20% polyacrylamide and 0.067% bis-acrylamide. After electrophoresis the gels were dried and exposed to X-ray film for 14 d for autoradiography. *a*, Infection with $\lambda\text{gt-}\lambda\text{C}'$; *b*, uninfected control; *c*, infection with $\lambda\text{gt-}\lambda\text{C}'$. The molecular weight scale given on the right (in kdaltons) is based on the following standards: serum albumin, catalase, ovalbumin, γ globulin (two chains), trypsin, cytochrome *c*, and chymotrypsin (two chains). Identification of protein bands, except for the products of the late genes *E* and *V*, is based on the combined use of deletions and amber mutations. The discrepancy in molecular weight determination of *Int*, *Ea22* and *Ea10* with respect to published data is due to the difference in gel composition (my work, in preparation).

operon, whose transcription is initiated at the P_L promoter (Fig. 1). But in addition to P_L -promoted transcription, the *int* gene is specifically activated by the action of the *cII* and *cIII* gene products, and we have proposed that the *cII* and *cIII* proteins stimulate fast synthesis of *Int* by acting at the P_L promoter (my unpublished work with M. Katzir, M. Belfort and A. Oppenheim). In this respect the *int* gene behaves similarly to the *cI* repressor gene, whose fast expression is activated by the gene products of *cII* and *cIII*^{2,3}.

Strains of λ have been constructed by rejoining *EcoRI* DNA fragments^{4,5}. As cohesive ends generated by the *EcoRI* nuclease are symmetrical, in a reconstructed phage a DNA segment, not essential for phage growth, can be found either in the native or inverted form. $\lambda\text{gt-}\lambda\text{C}'$ is such a phage in which the *att-int-exo* region has been inverted⁶ (Fig. 1). A gene within the inverted segment would be expected to express itself only if its promoter is also included within the inverted region. On the other hand, if the promoter of the gene in question is outside the inverted segment only "anti-sense" message will be transcribed and no functional protein will be produced. I have used this principle to define the location of the promoter for fast expression of the *int* gene.

Ultraviolet irradiated cells were infected by either $\lambda\text{gt-}\lambda\text{C}$ or $\lambda\text{gt-}\lambda\text{C}'$, then pulse labelled with ¹⁴C and subjected to electrophoresis as described in the legend to Fig. 2. Representative autoradiograms are shown in Fig. 2. Evidence for the identification of the various protein bands will be published elsewhere.

The results presented in Fig. 2 can be summarised as follows. First, the synthesis of the proteins coded by the genes *bet* and *Ea10* (Fig. 1) was unaffected by the presence of the inverted segment (Fig. 2). Protein band *Ea22* was present in the native phage (Fig. 2a) but absent from the inverted phage (Fig. 2b). This result was expected as the gene which is responsible for the synthesis of this protein band is present in the inverted segment. Second, the two phages show a burst of synthesis of both the *cI* repressor (26,000 molecular weight band) and *int* gene product (36,000 molecular weight band). This suggests that the inverted segment containing the *int* gene includes also its promoter for fast expression. Third, several of the major late protein bands (*E*, *V* and others) can be recognised in Fig. 2. None of these proteins is affected by the inverted segment. Fourth, a protein with a molecular weight of 31,000, designated *X*₁, is synthesised early after infection with $\lambda\text{gt-}\lambda\text{C}$ but not after infection with $\lambda\text{gt-}\lambda\text{C}'$. This protein band is not present in

infections with λ^+ , nor has it been observed in a large number of phage mutants screened in our laboratory. We do not know the origin of this protein band nor why it is not synthesised in $\lambda\text{gt-}\lambda\text{C}'$. It may result from a genetic fusion in the construction of the phage, probably at the *R1-2* site.

These results indicate that fast expression of the *int* gene originates at a promoter located within the *att-exo* region and is independent of transcription from P_L .

I thank Dr Ronald Davis for phage strains; Dr A. Oppenheim, Mrs N. Katzir, Mrs N. Kass and Miss S. Abergail for help. This work was supported by a contract with the Abteilung für Molekular Genetik der Gesellschaft für Strahlen und Umweltforschung Göttingen and the United States-Israel Binational Science Foundation.

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reviews

Mustard seed of mystery

Michael W. Ovenden

The Sirius Mystery. By Robert K. G. Temple. Pp. viii+290+21 plates. (Sidgwick and Jackson: London, 1976.) £6.95.

THE Dogon of west Mali are an artistic people, whose sculptures had a strong influence on Picasso and Braque. Two French anthropologists, Marcel Griaule and Germaine Dieterlen, lived for a time with the Dogon, and (by special permission of the priests assembled) Marcel Griaule was initiated into some of their most secret knowledge.

There is a strong astronomical flavour to some of this esoteric tradition. For example, the Dogon know of the four bright satellites of Jupiter (which are, however, just visible to an acute and attentive human naked eye). But the Dogon also describe a star companion to Sirius. They name this star after the smallest seed known to them, translated by Griaule to its Latin form *Digitaria*. *Digitaria*, say the Dogon, is the smallest and heaviest thing in the sky, and it moves around Sirius with a period of 50 yr. Now Sirius B is a white dwarf—and its period about Sirius is given (Aitken, 1932) as 50.04 ± 0.09 yr. Yet the visual magnitude of Sirius B is about 8.7; Sirius B is totally invisible to even the most acute human eye. This is the Sirius Mystery—the starting-point for Robert Temple's fascinating, frustrating and ultimately sad book.

In approaching the mystery with that open-minded scepticism which is the hallmark of (ideal) science, we might wonder whether the reporting of the tradition has been influenced by the astronomical knowledge of the reporters. The internal evidence is against this. The anthropologists seemed quite unaware of the extraordinary nature of their discovery, or how accurately the Dogon drawings of the path of *Digitaria* match the apparent (projected) orbit of Sirius B about Sirius. Can we postulate some astronomically-minded colonial administrator or seeker of artefacts who put, for the Dogon, a twentieth-century gloss on their ancestral astral traditions? Unlikely—for the behaviour of *Digitaria* plays a significant, albeit obscure, part in the chronology of religious ceremonies that clearly go back several hundred years, to long before Alvan



The four Dogon priests who revealed the Sirius traditions to anthropologists (Top: Manda D'Orosongo and Innekouzou; bottom: Ongnonlou and Yébéné)

Clark 'discovered' Sirius B in 1862 (using an 18.5-inch refracting telescope). And how could our hypothetical astronomer explain Dogon statements about the Sirius system that are *not* part of present astronomical understanding—such as the 'fact' that *Digitaria* is brighter when it is nearer to Sirius, a proximity effect which is neither observed now nor explicable by present ideas, in such a wide system as that of Sirius.

How came the Dogon on such strange knowledge? Robert Temple takes the view that the Dogon traditions are part of an earlier tradition from elsewhere. He devotes much space to the origins of the Dogon, whom he links with the Garamantes of Herodotus, and ultimately with the Argonauts. Maybe! But to explain the survival with the Dogon of ancient tradition we need only recall that, in the sixteenth century, at Timbuktu in Mali, there flourished a leading university of the Muslim world. Through Timbuktu would have flowed

the traditions of Greece, Egypt and Sumer.

At this point, Robert Temple sets off on a scramble through classical literature and Egyptian cosmology, seeking hints of the same Sirius mythology. There is much to be enjoyed here—especially the byways of Proclus and the neoplatonists, whose influence on the development of modern science is only just being realised. But (for example) to treat every reference to the number '50', from the oars of the Argonauts to the weight of the armour of Gilgamesh (50 minas) as sublime references to the period of Sirius B, fails to carry conviction with me. The tradition is supposed to be esoteric—but if it is as esoteric as that, we cannot unscramble it.

All this simply relocates the mystery in time and place; it is still a mystery. But not to the Dogon. In their mythology, *Digitaria* and the Sirius system are sacred, because from these came the Nommo—fish-like creatures who instructed mankind in the arts of civil-

isation. Of course, Robert Temple sees at once a connection between the Nommo of the Dogon and Oannes and the Annetodus of the Chaldeans; he kindly reprints for us in English translation the accounts of Oannes from Berossus *et al.* Oannes came from the Erythraean Sea, not from Sirius—so far so good. The author goes on to suppose, however, these myths to be literal statements of historical truth. Is this reasonable? The answer is—we simply don't know. Robert Temple is not the first non-astronomer to be misled by calculations of the 'probability' of life on other worlds, which are simply the prejudices of the respective writers dressed up in a spurious numerical precision. Certainly the idea of intelligent visitors from space is not *absurd*. How much evidence one would require before embracing the hypothesis tells more about one's psychology than it does about the historical problem.

This is a fascinating book because the nugget of mystery that the author has mined and polished is from a pure vein. The book is frustrating because the arguments are loose, and much of what the author has written is only marginally connected with his theme. A shorter, tighter book would have made more impact.

Ultimately, the book is sad for two reasons. In the first place, Robert Temple attempts to dissociate himself

from "... the people who will enthusiastically receive my researches with open arms (but who are) the sort of people one least wants to be classed with". The author brings to his work a scholarship in oriental studies, and a belief in it that is transparently honest. The book should be taken seriously. Nevertheless, when all has been said and done, he (like others before him) is building a pyramid of speculation on a mustard seed of mystery. Such speculation can be judged only by its fruits. How often, since first learning of these ideas, have I been able to say, in my historical reading, "ah yes, that fits in so well." The answer is, not once; but perhaps I have been reading the wrong works.

This book is sad to me also in a different and deeper way. By taking myth only as history, we demythologise it. Myths (so I believe) express truths about the human predicament that cannot be expressed except in mythopoetic languages, in whose very ambiguity lies a richness of understanding. To take myth as history is to reduce it. It may be so. But I would rather my gods danced still on Mount Olympus than merely to have leased a country house in one of the more desirable subdivisions of Thessalia. □

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Vision in children

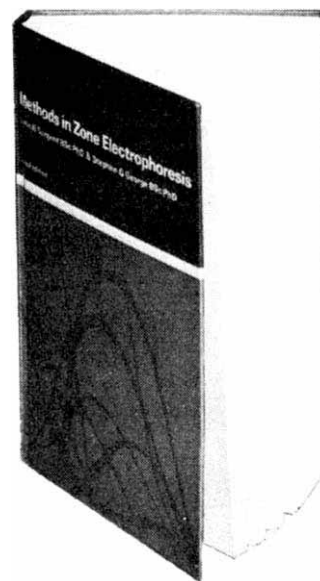
The Visual World of the Child. By Eliane Vurpillot. Pp. 372. (Allen and Unwin: London, April 1976.) £9.50.

If 4-yr-old children are presented with two panels bearing a square and a circle, they can learn to select the square consistently to obtain delivery of a sweet. If the experimenter then makes the sweet contingent on their pressing the circle, they normally take longer to learn this 'discrimination reversal' than they took to learn the original task. There is a story that as reversal training was about to begin one experimenter accidentally knocked over the apparatus that presented the stimuli and rewards: he restored it to an upright position, remarking that he hoped it was not broken. The child learned the reversal in a single trial. The story illustrates the extent to which children's behaviour may be governed by their expectations of how a piece of apparatus will behave and of how experimenters themselves are likely to behave. Such considerations have largely been ignored by the long list of psychologists whose work is summarised in *The Visual World of the Child*.

Disappointingly few secure or interesting conclusions emerge from Mademoiselle Vurpillot's catalogue raisonnée of experiments. Many of the theoretical issues raised are in any case so ill formulated or so trivial as to preclude the possibility of interesting answers. A question of the former sort is whether the child proceeds from 'synergetic' to 'analytic' vision—no-one knows; one of the latter sort is whether a child's eye movements become more systematic with age—they do. Research on visual development, particularly in Europe, still suffers from Piaget's obfuscations and some of the master's vague concepts, like 'centration' and 'assimilation' have proved singularly unhelpful when applied to perception, but are unfortunately still taken seriously. Moreover, the plethora of contradictory experimental results in this area stems in part from the difficulty in replicating many of his experiments.

Although the book comes with the good psychologist's seal of approval, provided in an encomium written by Professor Jerome Bruner, it will be useful mainly to those who would like some signposts through the morass of the European literature on vision in children. N. S. Sutherland

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Excited states

The Excited State in Chemical Physics. Vol. 28. Edited by J. Wm. McGowan. (Advances in Chemical Physics.) Pp. x+492. (Wiley/Interscience: New York and London, October 1975.) £13.75.

THIS recent addition to this well established series is planned as the first of two volumes intended to cover the properties and reactions of excited states of atoms and simple molecules. In it several experts critically review various aspects of the physics and chemistry of mainly neutral excited species. The first and longest chapter, by I. W. A. Smith, on the production of excited species in simple chemical reactions, provides a useful introduction, with almost 500 references, to molecular beam studies of reaction dynamics and to spectroscopic methods for studying excited species. R. C. Amme discusses equally well theory and experimental techniques concerning vibrational and rotational excitation in gaseous collisions, and J. J. Kaufman clearly demonstrates, in her contribution, the usefulness of potential energy

surface considerations in elucidating the detailed mechanisms for reactions which involve electronically excited states.

There is a chapter entitled 'Theory of Non-adiabatic Collision Processes including Excited Alkali Atoms' by E. E. Nikitin, whereas the emphasis is mainly experimental in the article on 'Sensitized Fluorescence and Quenching' by L. Krause. The final two short chapters are interesting accounts of applications to lasers by R. H. Bullis and of 'Excitation and De-excitation Processes Relevant to the Upper Atmosphere' by J. W. McGowan, R. H. Kummeler and F. R. Gilmore.

It is a pity that delays in publication dates have caused some of the chapters first written in 1969 to be redrafted later and to include supplements written in 1974. In rapidly expanding areas such as those discussed, this kind of delay means that the reviews and literature surveys become rapidly outdated. Although this is not of supreme importance for the non-specialist for whom this volume is mainly intended, it is nevertheless a disadvantage.

F. Wilkinson

Cell membranes

The Molecular Biology of Cell Membranes. By Peter J. Quinn. Pp. x+229. (Macmillan: London and Basingstoke, 1976.) Hard covers £8.95; paperback £3.95.

THIS book has developed partly from a course given to undergraduates and is aimed at "all students of the biological sciences who have completed a first course in cell biology or biochemistry" and "teachers and research workers in the field". I suspect that it will prove more useful to the latter group. In five chapters it covers Characterisation, Physical Structure and Molecular Organisation of Membranes, Membrane Permeability and Transport and Regulation of Cellular Processes by Membranes. Its particular emphasis, except in chapters 1 and 5, is on biophysical studies of model membrane systems and their relevance to the function of biological membranes. Sometimes this emphasis is excessive and one wonders whether Molecular Biology is an appropriate title—for example, we are told how liposomes and asymmetric black lipid films are made, but not how to isolate biological membranes. Biology only dominates chapter 5, and plants and prokaryotes hardly get a mention.

The format is largely textual, with only a minority of the illustrations of an interpretive nature, and most undergraduates will probably learn relatively

little from the frequent reproduction, without full interpretations, of original research data. The text itself is quite good, although it would be better if it was considerably more concise and carefully organised. There is a failure to cross reference apparently contradictory statements (for example, chapters 1 and 2 disagree on the significance of the amino acid compositions of intrinsic proteins, and some qualifying comment should accompany the separate statements that endoplasmic reticulum possesses 15 polypeptides and 75 enzymes). Some items are idiosyncratic, such as the description of peptide ionophores as proteins and the description of erythrocyte proteins mainly by reference to a single paper by Tanner and Boxer. Others are wrong—for example, gangliosides without sialic acid, the equating of glyceryl ethers with plasmalogens and of polypeptides separated by electrophoresis in SDS with proteins, secretion of digestive enzymes by pancreatic α cells, and the statement that the permeability of lipid bilayers to water is 8–9 times greater than to sugars and ions. All in all, the book may be useful to the well-informed and critical teacher looking for up-to-date and detailed information on model membranes, but it is neither balanced enough nor accurate enough for use as a main undergraduate text on biological membranes.

Robert H. Michell

Plumbing the depths

Underwater Research. Edited by E. A. Drew, J. N. Lythgoe and J. D. Woods. Pp. viii+430. (Academic: London and New York, March 1976.) £14; \$34.75.

ALTHOUGH so much less spectacular, the gradual conquest of the ocean depths has certainly been more immediately productive and at vastly lower cost than the conquest of outer space. What began largely to further biological observation has assumed impressive dimensions in the present search for oil, to be followed by that for mineral reserves.

This composite book, containing no less than 25 contributions, gives a first impression of over-diversity but this is in some measure alleviated when it is found that the first three papers represent almost half the contents. These deal with such fundamental matters as communication between, and hearing in, divers (Hollien and Rothman; Hollien and Feinstein) and problems of underwater vision (Cocking). These are written by active workers and bring us up to date (at the time of writing) with the progress in what are rapidly developing technologies. Increasing improvement here is essential if divers are adequately to co-operate and to observe in this alien environment.

Most of the remaining articles were originally contributed to the annual symposia of the Underwater Association. The first are concerned with aspects of underwater physiology such as the effects of narcosis and the measurement of respiration at high pressures. There is concern with underwater habitats, with surveying on the seabed and with quantitative estimations of underwater benthos.

The last lead on to straight zoological communications dealing with the crown-of-thorns starfish and the British cup coral, *Caryophyllia*. Botanists will find interest in work on submerged freshwater macrophytes and on the growth and physiology of *Posidonia* at Malta and of *Laminaria* in Scotland. I was particularly interested in R. K. Trench's admirably brief review of calcification in reef-building corals, although, with delays imposed by this form of publication, no longer quite up to date. The volume ends with an account of archaeological evidence for sealevel changes in the eastern Mediterranean.

One sees how wide the net has been cast; there is much here to interest all concerned with underwater activities and the book can be recommended for inclusion in libraries that are available to them.

C. M. Yonge

Taxonomy and medical aspects of amoebae

Pathogenic and Non-Pathogenic Amoebae. By B. N. Singh. Pp. ix + 235. (Macmillan: London and Basingstoke, October 1975.) £15.

DR SINGH'S book is divided into two sections—'Aerobic' and 'Anaerobic'. 'Anaerobic amoebae' is devoted to a full and useful review of work (much of it carried out in India) on the cultural requirements and pathogenicity of the dysentery amoeba. The problem of encystment is dwelt on, and methods of killing the cysts while still within the patient are discussed. Encystment and excystment factors are examined, mainly by using aerobic amoebae as model organisms. 'Aerobic amoebae' covers the amoebae of soil and water, and types of particular interest, taxonomically and medically are described in detail.

Confusion may arise in many minds when the taxonomic assignments of Singh and of the occidental authority, F. C. Page, are compared. Page considers, justifiably, I think, that Singh's *Hartmannella* comprises the two genera *Hartmannella* and *Acanthamoeba*. All pathogenic strains of *Hartmannella* (Singh) so far isolated would fit into Page's *Acanthamoeba*. There is a further conflict between Singh and Page over the existence of the genus *Didascalus* which Page would incorporate in the genus *Naegleria*. *Didascalus* as described by Singh has a 'promitotic' cell division without interzonal body formation, and is capable, with some difficulty, of producing a flagellate stage. *Naegleria*, according to Singh, invariably produces interzonal bodies during nuclear division; but, according to Page, this is not a consistent character. In the reviewer's experience, although the 'promitotic' division of *Naegleria* does not invariably reveal interzonal bodies, it generally does, and it seems sensible to separate *Didascalus* which never does, from *Naegleria* which generally has the interzonal bodies and is readily capable of forming flagellates.

A third point of conflict is linked to the preceding one, and is concerned with the nomenclature of amoebae with 'promitotic' division which do not produce a flagellate stage. Decision on this point is much more confusing, as it depends on interpretation of old descriptions in the literature, which are not even certainly of pure cultures. It is however, sufficient to say that the genus termed *Schizopyrenus* by Singh is in

all respects that termed *Vahlkampfia* by Page.

There is a fourth point in which Singh differs from what seems to be current terminology. He argues that the pathogenic HBI strain of *Naegleria* he initially studied is the same species as that isolated and studied by the Australian workers (This is supported by independent work.) R. F. Carter named the Australian organism *N. fowleri* in a publication slightly predating the Indian workers' description and naming of HBI as *N. aerobia*. After a rather tortuous argument, Singh claims that *N. aerobia* has priority over *N. fowleri*.

The foregoing points of taxonomic confusion serve to emphasise the need for the objective taxonomic criteria of a biochemical and immunological nature which are in the course of emerging in this rapidly expanding field.

The book is well produced and easy to read. It should form a useful introduction to the subject for the student or the research worker.

D. C. Warhurst

Immunosuppressive agents

The Mode of Action of Immunosuppressive Agents. (North-Holland Research Monographs: Frontiers of Biology, Vol. 41.) By Jean Francois Bach. Pp. xviii + 379. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1975). Dfl. 98; \$40.95.

THIS book is intended for the clinical immunologist who is involved with the use of immunosuppressive agents in man. Rather than review all the data related to immunosuppressive agents, for which there is admittedly scattered and incomplete information, the author has chosen to discuss four classes of agents in detail: corticosteroids (hydrocortisone and its derivatives), thiopurines (6-mercaptopurine and azathioprine), alkylating agents (cyclophosphamide) and anti-lymphocyte serum.

In the first chapter (general introduction) several important considerations related to immunosuppressive agents are discussed. This is a good beginning and provides the proper perspective for the subsequent chapters. The problems of definition of an immunosuppressive agent, anti-inflammatory effect, therapeutic index, drug evaluation and the experimental approaches to investigating a given drug are reasonably well covered.

The outline of the subsequent chapters are quite good and begin with the known chemistry of the agents, their pharmacology, toxicity, and so on, and proceed through a review of the effects of the agents on various aspects of the immune response, including clinical trials. Unfortunately, the promise provided by the first chapter and the logic of the outline of the subsequent chapters is not fulfilled by the text material.

Often the summarised reports represent a cataloguing of data and not enough critical evaluation is summarised by the author. The result will tend to confuse the new reader. A more serious objection is the lack at times of emphasising important findings that may have exciting implications. For example, cyclophosphamide may prevent allergic encephalomyelitis or thyroiditis but it will also produce a 'tolerant' state. The latter aspect is not discussed nor even noted.

Some of the review is quite outdated (for example, metabolism of cyclophosphamide) but more seriously there are a number of errors in reporting the literature as well as uncritical reporting. On p184, for instance, the author states that bone marrow grafts must be carried out 24 h after cyclophosphamide injection, because of the effects of residual cyclophosphamide on engrafted cells. This is true for the rat but certainly not true for the mouse. On p203, under the heading of tumor immunity, it is stated that cyclophosphamide may favour the development of L1210 leukaemia in C57BL mice. What is meant most certainly is that L1210 can be transplanted to a C57BL/6 mouse given cyclophosphamide. What does this have to do with tumour immunity, as this is an allograft!

The reference quoted is wrong and the data not accurately reported in several places in the chapter on alkylating agents.

Chapter 6 contains concluding remarks and guidelines for the clinical use of immunosuppressive agents. It is also disappointing, particularly in the choice of dosage and monitoring of the effects of a given agent on a disease process. In treating a disease process it would seem wisest to decide what clinical effects one desires and then treat the disease with doses of agents designed to give acceptable clinical toxicity. The acceptable toxicity, of course, will be dictated by the severity of the disease being treated. The author does not emphasise this philosophy but instead discusses *in vitro* monitoring of immunological function.

In view of the serious criticisms listed above, I cannot recommend this book to those interested in immunosuppression or immunology.

George W. Santos

obituary

Carl Peter Henrik Dam died in April 1976. He was born at Copenhagen in 1895 and educated there. He went to Graz (1925) to be trained in micro-analysis under Pregl and later worked with R. Schoenheimer at Freiburg (1932–33) and with P. Karrer at Zurich. He always tried to keep up his skill as a chemist while steadily widening his biological interests.

Early in his career (1929) Dam made the first observations that led by stages to the discovery of vitamin K and other 'bioquinones' and towards the end of his career his friends delighted to remind him "How far that little candle throws its beams".

He began by testing a claim that chicks could not thrive on an almost sterol-free diet. Some of his birds unexpectedly displayed haemorrhages under the skin, in muscles or other tissues, and the coagulation of their blood was much retarded. McFarlane in Toronto confirmed this. Dam's research was delayed by his visit to Freiburg, but on his return he and Schönheyder found that the chick symptoms were not due to sterol deprivation but to a dietary lack of anti-haemorrhagic vitamin from plants, first designated vitamin K and now known as phyloquinone. A chick bioassay was used to monitor the separation of lipid factors from alfalfa. Dam joined forces with Karrer in isolating and characterising the vitamin which was recognised (both at Zurich and in the USA) as 2-methyl-3-phytyl-1,4-naphthoquinone. With the methods then available this was a considerable feat. Phyloquinone is, quantitatively, a minor constituent of the quinones present in chloroplasts, and although the bio-assay successfully led to the vitamin, the more plentiful quinone congeners were missed.

It was found in the USA that putrefaction of ether-extracted fish meal changed it from a very poor to a very potent source of vitamin K activity. The formation of vitamin K by bacteria was followed up by Almquist and

Stokstad and by Doisy's group. In addition to isolating phyloquinone they obtained a second vitamin K (at first known as vitamin K₂ but now as a menaquinone). The new form was a 2-methyl-3-polyprenyl-1,4-naphthoquinone which later turned out to be one member of a large family of menaquinones denoted MK-*n*, where *n* is the number of prenyl (C₅H₈) groups in the polyprenyl sidechain.

Vitamin K activity was also found in a number of simpler but related substances (for example 2-methyl-1,4-naphthoquinone, or menadione, and numerous esters of derived quinols). These substances were seen as provitamins K. A new facet to the larger problem was Link's isolation and characterisation from spoiled sweet clover hay of dicoumarol, a specific vitamin K antagonist. The number of antagonists is now large, some of the best-known being dicoumarol, Warfarin, phthiocol, sulphaquinoxaline and actinomycin D.

The coagulation of blood is envisaged as a complex but ordered succession of processes, and at least four of the many factors (prothrombin, proconvertin, Christmas factor and Stuart factor) are known to be dependent on vitamin K. Of these the deficient chick lacks only the Christmas factor. Dam's group studied neonatal bleeding disorders, and advocated the administration of vitamin K to mothers a few hours before parturition. They also noticed that cows' milk is much richer in vitamin K activity than human milk.

In 1940 Dam went on a lecture tour in the USA and Canada and then became a Senior Research Associate at the University of Rochester (1924–45) returning to Denmark in 1946. In 1943 Dam and E. A. Doisy shared the Nobel Prize for Physiology and Medicine. While still in the USA Dam had been appointed to the Chair of Biochemistry and Nutrition at the Copenhagen Polytechnic Institute which he held until his retirement in 1965. He also played

a leading part in the work of the Danish Fat Research Institute (1956–63). He led a research school and published nearly 300 papers mostly concerned with cholesterol, vitamin E and vitamin K. His review articles were models of fairness and acumen as well as deep scholarship.

In 1966 the 4th Roche International Symposium was held at Elsinore in his honour. It was entitled 'Recent Advances in Research on Vitamins K and Related Quinones (Ubiquinones or Coenzymes Q and Plastoquinones)'. By that time the chemical diversity within the major families or bioquinones was evident and the central biological roles of quinones, quinols and chromanols had become as acceptable as they were surprising.

The 'prothrombin' synthesised by cattle given dicoumarol is not effective in blood coagulation and fails to bind calcium ions or phospholipids. Ten defined positions on the sequence 1–42 are occupied by glutamyl residues, whereas in normal prothrombin all ten are carboxylated to a previously unknown γ -carboxyglutamic acid. The vitamin K-dependent step in the formation of prothrombin is a post-synthetic γ -carboxylation of a precursor made in liver. Thanks to coalescing research from Denmark, Sweden, the USA and Britain, the principal role of vitamin K in the animal is perhaps now clear in the sense that what the vitamin does is established, if how it does it remains to be worked out.

At the symposium in his honour Dam was obviously gratified by developments arising from his original observations and it is very fitting that the recent work, entirely contemporary in its approach, should have come so near to rounding off his discovery.

Professor Dam travelled a good deal and was among the best known and most welcomed scientists in an ever-widening field.

R. A. Morton

announcements

Awards

The **William Bate Hardy Prize** of the Cambridge Philosophical Society has been awarded to **Dr F. Sanger, FRS**, of

King's College for his distinguished work on the sequencing of DNA.

The Société Française de Physique has made the following awards:

Le grand prix de physique Jean-Ricard to **Georges Slodzian** of the University of Paris-Sud (Orsay)

Le prix Robin to **Jacques Prentki** of the College de France

Le prix Ancel to **Gerard Jannink** of the Centre d'Etudes Nucléaires de Saclay

Le prix Aime-Cotton to **Michel Gaillard** of the University de Lyon

Le prix Paul-Langevin to **Gerard Toulouse** of the Laboratoire de Physique des Solides à Orsay

Le prix Esclançon to **A. Quinzer** of the Laboratoire de l'Accélérateur Linéaire d'Orsay

Le prix Foucault to **J.-P. Taran** of the Office national d'Etudes et de Recherches Aérospatiales.

International meetings

August 1-7, **High Speed Photography**, Toronto (Congress Secretariat, c/o Ontario Science Centre, 770 Don Mills Road, Don Mills, Ontario, Canada M3C 1T3).

August 2-3, **Rocky Mountain Spectroscopy Conference**, Denver (Ms Jean R. Gaines, 1012 4th St, Golden, Colorado 80401).

August 18-27, **Mathematical Geophysics**, Seeheim/Odenwald, FRG (Professor Karl Fuchs, Geophysikalisches Institut, Universität Karlsruhe, Hertz-Str. 16, 75 Karlsruhe, FRG).

August 19-27, **Entomology**, Washington DC (Dr Ernest C. Bay, Secretary General, XV International Congress of Entomology, PO Box 151, College Park, Maryland 20740).

August 22-27, **Liquid Crystals**, Kent, Ohio (Organising Committee, Liquid Crystal Institute, Kent State University, Kent, Ohio 44242).

August 22-28, **Transplantation**, New York City (Congress Secretariat, Felix T. Rapaport, New York University Medical Center, 560 First Avenue, New York, N.Y. 10016).

August 23-27, **Analytical Chemistry in the Exploration, Mining and Processing of Materials**, Johannesburg (The Conference Division (IUPAC Symposium), CSIR, PO Box 395, Pretoria, 0001, RSA).

August 27-29, **Forensic Toxicology**, Ghent (Professor A. Heyndrickx, Department of Toxicology, State University of Ghent, Hospitaalstraat 13, 9000 Ghent, Belgium).

August 29-September 3, **Invertebrate Pathology**, Kingston, Ontario (Dr Peter Faulkner, Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6).

September 22-24, **Pressure Surges**, London (The Organising Secretary, 2nd ICPS, BHRA, Fluid Engineering, Cranfield, Bedford MK43 0AJ, UK).

Person to Person

The annual introductory course on tropical hygiene will be held at the Ross Institute this year from July 12-16. The course is designed for para-medical and non-medical people proceeding to the tropics, especially for those who may be responsible for the health of others, and is directed towards giving a comprehensive understanding of the prevention of tropical diseases caused by infection, insanitation and malnutrition. There is no fee for the course. Recognising its value, many companies and institutions with interests in the tropics pay the expenses of their staff during their stay in London, and it is hoped that this practice will become general. Contact: Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London WC1E 7HT.

Professor A. K. Som of University of Malawi, Box 280, Zomba, Malawi, on sabbatical leave, wishes to rent a centrally heated furnished 2/3 bedroom house or flat in London from August '76 to Sept. '77. Please refer to Dr G. Leventhall, Physics Dept., Chelsea College, London.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

November 19, **X-Ray Diffraction**, Wembley (Abstracts by October 1 to Dr B.J. Isherwood, GEC Hirst Research Centre, East Lane, Wembley, Middlesex HA9 7PP), (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

February 28-March 4, 1977, **Analytical Chemistry and Applied Spectroscopy**, Cleveland, Ohio (Deadline for abstracts: September 15), (John E. Graham, Program Chairman, Koppers Company, Inc., 440 College Park Drive, Monroeville, Pennsylvania 15146).

July 26-29, 1977, **Electron Transport/Molecular Solids**, Leeds (Preliminary offers of papers by October 1 to Professor G. J. Morgan, Department of Physics, University of Leeds, Leeds LS2 9JT, UK), (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

Reports and publications

Control of the Noise and Vibration Environment. By Professor P. Grootenhuys. (Inaugural Lecture, 12 November 1974.) Pp. 15. £1.10. Complex Fluids in Engineering. By Professor J. R. A. Pearson. (Inaugural Lecture, 3 June 1975.) Pp. 37-444. 90p. (London: Imperial College of Science and Technology, University of London, 1974 and 1975.) [103]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 281, No. 1301: The Symmetry of Electron Diffraction Zone Axis Patterns. By B. F. Buxton, J. A. Eades, J. W. Steeds and G. M. Rockham. Pp. 171-194 + plates 1-3. (London: The Royal Society, 1976.) UK £1.75; Overseas £1.80. [113]

Royal Observatory Annals. No. 10: Photoheliographic Results 1966. Pp. 67. (Herstmonceux: Royal Greenwich Observatory, 1974.) [113]

Energy Audits. (Fuel Efficiency Booklet, No. 1.) Pp. 12. (London: Library, Department of Energy, Thames House South, Millbank, SW1, 1976.) grat. [123]

Bulletin of the British Museum (Natural History). Entomology. Vol. 32, No. 6: A Revision of the Genus *Ptychandra* (Lepidoptera: Nymphalidae). By H. J. Banks, J. D. Holloway and H. S. Barlow. Pp. 215-252 + 5 plates. (London: British Museum (Natural History), 1976.) £4.25. [123]

Science and the Media-Report of a Study Group. (British Association Publication 76/1.) Pp. 27. (London: British Association for the Advancement of Science, 1976.) 50p. [123]

Male and Female Sterilization. Second edition. Edited by R. L. Kleinman. Pp. 39. (London: International Planned Parenthood Federation, 18/20 Lower Regent Street, SW1, 1976.) 80p; \$1.75. [153]

Bulletin of the British Museum (Natural History). Botany. Vol. 5, No. 5: Frank Ludlow (1885-1972) and the Ludlow-Sherriff Expeditions to Bhutan and South-Eastern Tibet of 1933-1950. By W. T. Stearn. *Reliquiae Botanicae Himalaicae*. By Frank Ludlow. Pp. 241-289 + plates 30-36. £4.75. Entomology. Vol. 32, No. 5: An illustrated List of the Type-Specimens of the Heliconiinae (Lepidoptera: Nymphalidae) in the British Museum (Natural History). By P. C. Ackery and R. L. Smiles. Pp. 171-214 + 39 plates. £10.70. Geology. Vol. 26, No. 6: Dinoflagellate Cysts from the Brackishwater Beds (Eocene) of the Isle of Wight, Southern England. By G. L. Eaton. Pp. 225-332 + 21 plates. £11.60. (London: British Museum (Natural History), 1976.) [153]

Bedfordshire Plant Atlas. By John G. Dony. Pp. 132. (Luton: Borough of Luton Museum and Art Gallery, 1976.) £3. [153]

Register of Consulting Scientists, Contract Research Organizations and other Scientific and Technical Services. Third edition. Pp. 137. (Stoke Poges, Slough: Hon. Registrar, Fulmer Research Institute, Ltd., 1976.) £7 + 50p. postage and packing. [153]

The Association of Commonwealth Universities. Annual Report of the Council, 1974-75. Pp. 50. (London: The Association of Commonwealth Universities, 1976.) [163]

Engineering Design Education. (A Report on the Current Education in Engineering Designers in Britain with Recommendations for Future Policies and Action.) Pp. 12. (London: Design Council, 1976.) 50p. [173]

Natural Environment Research Council. International Hydrological Decade. Hydrological Research in the United Kingdom (1970-1975). Pp. vi + 180. (London: Natural Environment Research Council, 1976.) [173]

Computers and Chemistry. Vol. 1, No. 1. Pp. 1-68. Annual Subscription Rates for 1976: For Libraries, Research Establishments and all other Multiple-Reader Institutions \$60. (Oxford and New York: Pergamon Press, 1976.) [183]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 274, No. 929: The Structure and Function of Thoracic Exopodites in the Larvae of the Lobster. *Homarus gammarus* (L.) By D. M. Neil, D. L. Macmillan, R. M. Robertson and M. S. Laverack. A Quantitative Analysis of Exopodite Beating in the Larvae of the Lobster *Homarus gammarus* (L.) By D. L. Macmillan, D. M. Neil and M. S. Laverack. A Comparison of Beating Parameters in Larval and Post-Larval Locomotor Systems in the Lobster *Homarus gammarus* (L.) By M. S. Laverack, D. L. Macmillan and D. M. Neil. Pp. 53-99. UK £2.50; Overseas £2.60. Vol. 274, No. 930: The Nervous System of *Loligo*. II. Suboesophageal Centres. By J. Z. Young. FRS. Pp. 101-167 + plates 1-10. UK £5.30; Overseas £5.45. (London: The Royal Society, 1976.) [193]

Shirley Institute, Report and Accounts, 1974/1975. Pp. 20. (Didsbury, Manchester: Shirley Institute, The Cotton Silk and Man-made Fibres Research Association, 1976.) [193]

Department of Trade. Report by the Department of Trade on the action taken to deal with the oil spilled as a result of the collision in the Dover Strait on 12 November 1975 between the M/T Olympic Alliance and HMS Achilles. Pp. 20. The Battle Against Oil Pollution at Sea. Pp. 12. (London: Department of Trade, Obtainable from The Marine Library, Sunley House, 90/93 High Holborn, London, WC1.) [193]

Review of the Water Industry in England and Wales: a Consultative Document. Pp. 33. (London: Department of the Environment, 1976.) [193]

The British Library. Research and Development Reports. Report No. 5233: British Library Working Party on Classification and Indexing. Pp. 25. £2; \$4. Report No. 5256: Inventory of Bibliographic Data Bases Produced in the UK. Pp. 82. £2.50; (Boston Spa, Wetherby: Publications Department, The British Library Lending Division, 1975 and 1976.) [193]